AD ________________

GRANT NUMBER: DAMD17-96-1-6035

TITLE: Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity

PRINCIPAL INVESTIGATOR: David H. Overstreet, Ph.D.

RECIPIENT ORGANIZATION: University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-4673

REPORT DATE: July 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinion and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
July 1997

3. REPORT TYPE AND DATES COVERED
Final (1 Jul 96 - 30 Jun 97)

4. TITLE AND SUBTITLE
Effects of Pyridostigmine in Flinders line Rats Differing in Cholinergic Sensitivity

5. FUNDING NUMBERS
DAMD17-96-1-6035

6. AUTHOR(S)
David H. Overstreet, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-1350

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The Flinders Line rats were developed at Flinders University in Australia by selective breeding for differential responses to the anticholinesterase, diisopropyl fluorophosphate (DFP). Subsequently, it was determined that the FSL rats were also more sensitive to directly acting muscarinic agonists as well as a variety of other drugs, including alcohol, diazepam, nicotine. The heightened sensitivity of the FSL rats to a variety of drugs suggests that they will also be more sensitive to the effects of pyridostigmine, an anticholinesterase which was given to gulf war participants. The results of initial experiments indicate that there are no line differences in telemetrically monitored hypothermia or general activity after pyridostigmine (4, 12, or 36 mg/kg, orally administered), even though there were substantial differences in hypothermia induced by the muscarinic agonist, oxotremorine. These negative findings were expected because pyridostigmine does not exert central cheolinergic effects necessary to induce these changes. Growth hormone level, the parameter most likely to change after pyridostigmine treatment, is still being analyzed.

14. SUBJECT TERMS Gulf War Illness

15. NUMBER OF PAGES
72

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

______ Where copyrighted material is quoted, permission has been obtained to use such material.

______ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

______ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

DHO In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

______ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

______ In conducting research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

______ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

David Overstreet
July 30, 1997

TABLE OF CONTENTS FOR DAMD17-96-1-6035

Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity

INTRODUCTION

In the assessment of risk to individuals exposed to known or potential toxicological agents. There needs to be a consideration of the possibility that especially sensitive populations exist. For example, some individuals have reported side effects after taking pyridostigmine to protect them against potential nerve gas exposure and others have not. Other individuals have reported increased sensitivity to a variety of chemical agents, usually after a triggering exposure to a specific chemical such as an organophosphate pesticide (e.g., Miller and Mitzel, 1995). The hypothesis that a genetically based cholinergic supersensitivity might underlie the increased sensitivity of these vulnerable human populations will be addressed in the present communication by describing in detail the features of an animal model with cholinergic supersensitivity which is also more sensitive to a variety of drugs and other chemical agents and which may, therefore, mimic the human condition labeled Multiple Chemical Sensitivity (MCS). In the body of this paper results on the effects of pyridostigmine in this animal model will be presented.

Multiple Chemical Sensitivity

Multiple Chemical Sensitivity (MCS) is a syndrome in which, following acute or repeated exposure to one or more chemicals, most commonly organophosphate pesticides (OPs), individuals become overly sensitive to a wide variety of chemically-unrelated compounds. These can include ethanol, caffeine and other psychotropic drugs (Ashford and Miller, 1989, 1991; Bell et al., 1992; Cullen, 1987; Miller, 1994). The symptoms of MCS often reported include fatigue, cognitive difficulties, depression, irritability, headaches, dyspnea, digestive problems, musculoskeletal pain, and numbness in their extremities. These conditions often overlap those of common medical illnesses such as depression, somatization disorder, chronic fatigue syndrome, fibromyalgia, asthma and others.

However, a distinguishing feature of MCS is the strong belief of the patients that their symptoms are brought on by common exposures to low levels of volatile organic chemicals such as fragrances, insecticides, traffic exhaust, disinfectants and perfumes. A more comprehensive discussion of MCS is included in the accompanying manuscript (Overstreet et al., 1997; see Appendix).

An important observation in this field is that MCS patients usually report that other individuals simultaneously exposed to similar amounts of pesticides, e.g., family members, friends, or co-workers, did not develop MCS or even experience transient illness. This observation suggests that a subset or subsets of the people may be more vulnerable to developing MCS. Indeed, some (Black et al., 1990; Simon et al., 1990), but not all (Fiedler et al., 1992) researchers have reported greater rates of depression and somatization disorder predating the "initiating" chemical exposure among persons with MCS as compared to controls. Thus, any model must take into account why only some individuals develop MCS after exposures to pesticides or other chemicals.

The FSL Rat Model

One such model which will be described in the subsequent sections of this paper is the FSL (Flinders Sensitive Line) rat. This rat was developed by selective breeding for increased sensitivity to an OP, so it shares some etiologic similarity to patients with MCS who were exposed to pesticides. The FSL rat model is one with which we have had extensive experience, particularly in research on depressive syndromes (Overstreet, 1993; Overstreet and Janowsky, 1991; Overstreet et al., 1995). Analogies between depressed states and MCS, as well as substance hypersensitivities in FSL rats, first brought our attention to the potential value of this model for experimental studies of MCS, as recently described (Overstreet et al., 1996). Further, because the FSL rats were selectively bred for increased responses to the organophosphate, diisopropylfluorophosphate (DFP), it is possible that they may have

some special relevance to Gulf War Illness, commonly reported in individuals exposed to the carbamate, pyridostigmine.

Selective Breeding for OP Differences. The FSL rat model arose from a selective breeding program designed to produce two lines of rats, one with high (FSL) and one with low (Flinders Resistant Line - FRL) sensitivity to the anticholinesterase agent, DFP (Overstreet et al., 1979; Russell et al., 1982). The selective breeding program, which was initiated at Flinders University in Adelaide, Australia, utilized three somatic measures of DFP (Overstreet et al., 1979; Russell et al., 1992). A rank-order system was used to give equal weighting to each of the three variables. Rats which had the lowest average ranks were intermated to establish and maintain the line of more sensitive rats (FSL), while rats which had the highest average ranks were intermated to establish and maintain the line of more resistant rats (FRL). Subsequent studies showed that randomly bred Sprague-Dawley rats, from which the lines were originally derived, were not different from the FRL rats. On the other hand, FSL rats were significantly more sensitive to DFP than the other two groups (Overstreet et al., 1979; Russell et al., 1982).

Biochemical Mechanisms. This project was initiated, in part, to develop genetically resistant lines of rats so that the biochemical mechanisms of resistance could be compared with those of tolerance. Early studies ruled out changes in acetylcholinesterase as a mechanism to account for the differential sensitivity of FSL and FRL rats to DFP (Overstreet et al., 1979; Russell and Overstreet, 1987; Sihotang and Overstreet, 1983), just as has been found for tolerance development (See Russell and Overstreet, 1987). Because DFP-tolerant rats were subsensitive to the effects of muscarinic agonists (e.g., Overstreet et al., 1973, 1974), the effects of muscarinic agonists on the FSL and FRL rats were examined (Overstreet 1986; Overstreet and Russell, 1982; Overstreet et al., 1986a,b). These

studies showed that the FSL rats were more sensitive to pilocarpine, arecoline and oxotremorine than were the FRL rats; this supersensitivity was seen for a variety of responses, including hypothermia, reduced locomotor activity, and suppression of bar-pressing for water reward (Overstreet and Russell, 1982). Thus, FSL rats, developed by selectively breeding for increased sensitivity to DFP, exhibited opposite changes in sensitivity to muscarinic agonists compared to DFP-tolerant rats.

Biochemical studies indicated that the FSL rats exhibited greater numbers of muscarinic receptor binding sites in the hippocampus and striatum than the FRL rats (Overstreet et al., 1984; Pepe et al., 1988)., but there were no differences in acetylcholine turnover (Overstreet et al., 1984). Thus, once again, the FSL rats appear to represent the converse of DFP-tolerant rats; having increased numbers of receptors rather than reduced numbers (See Russell and Overstreet, 1987). It appears that both tolerance and acute sensitivity to cholinergic agents is related to postsynaptic cholinergic mechanisms rather than presynaptic. Although in both instances, there have been detectable changes in the muscarinic receptors themselves, there are some findings, such as the increased sensitivity of FSL rats to noncholinergic agents (See Section below), which suggest that post-receptor mechanisms may also contribute.

<u>Behavioral Features of FSL Rats</u>. The FSL and FRL rats differ on a large number of behavioral tasks, as recently summarized in several review papers (Overstreet et al., 1995, 1996). In this section we will highlight a number of the key differences. The FSL rats have been reported to have lower locomotor activity than the FRL rats under a number of experimental conditions (Bushnell et al., 1995; Overstreet, 1986; Overstreet and Russell, 1982) but not all (Criswell et al., 1994; Rezvani et al., 1994). They are even less active when stressed prior to exposure to the open field (Overstreet, 1986; Overstreet et al., 1989a).

Results from several other behavioral paradigms are consistent with the view that depressive-like psychomotor retardation symptoms are more apparent in the FSL rats after exposure to stressors. For example, the FSL rats are impaired in active avoidance paradigms compared to the FRL rats (Overstreet and Measday, 1985; Overstreet et al., 1990a, 1992a). Another stress-oriented paradigm which has provided important information about behavioral differences between FSL and FRL rats is the forced swim test. Upon initial exposure in a cylinder (18-20 cm diameter) of water (25 oC), FSL rats are more immobile than the FRL rats (Overstreet, 1986; Overstreet et al., 1986a, Pucilowski and Overstreet, 1993; Schiller et al., 1992). This exaggerated immobility of the FSL rats is counteracted by chronic but not acute treatment with antidepressants (Overstreet, 1993; Pucilowski and Overstreet, 1993; Schiller et al., 1992). These findings provide further support for the contention that the FSL rat is a useful animal model of depression.

There are also differences in reward-related behaviors between the FSL and FRL rats which are consistent with the proposal that the FSL rats are a model of depression. In operant bar-pressing tasks, the FSL rats bar-pressed at lower rates and had to be maintained at a lower percentage of their free-feeding body weight and have smaller food pellets (37 vs. 45 mg) in order to keep their motivation sufficiently high to complete the session (Bushnell et al., 1995; Overstreet and Russell, 1982)). Despite these differences in reward-related and stress-related behaviors, there appears to be no differences between the FSL and FRL rats in the ability to perform a matching-to-sample task (Bushnell et al., 1995). However, this test was carried out under normal, unstressed conditions, and it is not clear whether similar findings would be obtained under stressed conditions. For example, FSL and FRL rats have similar amounts of saccharin consumption under baseline conditions, but the FSL rats exhibit greater decreases after exposure to chronic mild stress (Pucilowski et al., 1993).

The FSL rats also have elevated REM sleep and reduced latency to REM sleep (Shiromani et al., 1988, Benca et al., 1996), as has been reported in human depressives (Benca et al., 1992). Human depressives are also more sensitive to the effects of cholinergic agonists on REM sleep latency (Janowsky et al., 1994), but there are no data in the FSL rats regarding drug effects on sleep..

In sum, the FSL rats and depressed humans exhibit a large number of behavioral and physiological similarities (See Overstreet, 1993; Overstreet et al., 1995, 1996, for more detailed accounts).

Multiple Chemical Sensitivity in FSL Rats. Clinical observations suggest that MCS may be initiated by acute or chronic exposure to a variety of chemical agents (Miller and Mitzel, 1995). Because the FSL rats were selectively bred to have increased responses to the anticholinesterase agent, DFP, it should not be surprising that they exhibited increased sensitivity to muscarinic agonists (Daws et al., 1991; Overstreet, 1986; Overstreet and Russell, 1982; Overstreet et al., 1992a,b; Schiller et al., 1988). It has also been reported that human depressives are also more sensitive to directly acting muscarinic agonists (Gann et al., 1992; Gillin et al., 1991) as well as anticholinesterases (Gann et al., 1992; Janowsky and Risch, 1987; Nurnberger et al., 1989; O'Keane et al., 1992; Schreiber et al., 1992; Sitaram et al., 1987). A similar increased sensitivity to anticholinesterases has been observed in MCS patients (Cone and Sult, 1992; Miller and Mitzel, 1995; Rosenthal and Cameron, 1991), but there are no published data for MCS patients regarding sensitivity to direct cholinergic agonists. FSL rats are also more sensitive to nicotine, which interacts with nicotinic cholinergic receptors (Schiller and Overstreet, 1993).

The cholinergic system interacts with many other major neurotransmitter systems, including serotonergic, dopaminergic, GABAergic, and noradrenergic. Having animals with clear-cut differences

in the cholinergic system afforded us the opportunity to test how the FSL and FRL rats differ in response to drugs interacting with these other neurotransmitter systems. Evidence from various drug challenge studies, in which relatively selective drugs are given to FSL and FRL rats, have revealed a substantial number of differences between the FSL and FRL rats, as summarized in Table 1. FSL rats were found to exhibit a greater degree of hypothermia after a variety of drugs which interact with the serotonin 5-HT1A receptor (Wallis et al., 1988; Overstreet et al., 1992a, 1994). This outcome is consistent with much of the evidence suggesting supersensitive serotonergic mechanisms in depressives (Arango et al., 1990; Arora and Meltzer, 1989; Mikuni et al., 1991), but is not consistent with neuroendocrine studies reporting blunted responses to serotonergic agonists, which suggests serotonergic hyposensitivity (Lesch et al., 1990; Meltzer and Lowy, 1987). There are no data on the effects of selective serotonergic agents in MCS patients, but there is one report of supersensitive responses in individuals with chronic fatigue syndrome, which is related to MCS (Backheit et al., 1992).

To date no evidence has been obtained to indicate any differences in responses to noradrenergic agents in the FSL rats (Overstreet, 1989; Overstreet et al, 1989a). In contrast, there are quite a number of differences with regard to dopaminergic agents (Table 1). The FSL rats are supersensitive to the hypothermic (Crocker and Overstreet, 1991) and aggression-promoting (Pucilowski et al., 1991) effects of apomorphine, a mixed D1/D2 agonist, and quinpirole, a selective D2 agonist. On the other hand, the FSL rats were subsensitive to the stereotypy-inducing effects of similar doses of the same compounds and there were no apparent differences in dopamine D2 receptors between FSL and FRL rats (Crocker and Overstreet, 1991). These opposite changes in sensitivity in the various functions might be related to the type of modulation of these functions by the cholinergic and dopaminergic systems. Stimulation of both cholinergic and dopaminergic systems promotes hypothermic and aggressive responses (Cox et al.,

1980; Pucilowski, 1987; Ray et al., 1989), but cholinergic stimulation reduces activity and stereotypy, thereby opposing the effects of dopaminergic stimulation (Fibiger et al., 1970; Klemm, 1989).

The FSL and FRL rats are differentially sensitive to the effects of several pharmacological agents which have modulatory roles at the GABA-A receptor, as summarized in Table 1. However, as with the case of dopamine agonists, the differential effects are observed only for some actions of the drugs, not for all. For example, the hypothermic effects of ethanol are significantly higher in the FSL rats compared to the FRL rats, but the sedative effects are similar (Overstreet et al., 1990b). Similarly, the behavioral suppressant effects of diazepam are significantly greater in the FSL rats (Pepe et al., 1988), but its anxiolytic effects in the two lines are comparable (Schiller et al., 1991). The fact that these two commonly abused psychotropic drugs modulate GABA function at the GABA-A receptor suggests that there might be differences in GABA-A receptor subtype composition between the two lines, but there is not biochemical evidence for such differences as yet. Furthermore, despite differences in sensitivity to the hypothermic effects of ethanol, the FSL and FRL rats do not differ in their rates of voluntary ethanol consumption (Overstreet et al., 1992a).

In summary, it appears that the FSL rat is more sensitive to a variety of chemical agents in addition to the OP anticholinesterase agent for which they were selectively bred. In this regard, the FSL rat is somewhat analogous to MCS patients who have become more sensitive to a range of agents following exposure to OP anticholinesterases. The extent of the similarity between the FSL rats and MCS patients, on one hand, and human depressives and MCS patients, on the other, has been more extensively evaluated in the accompanying manuscript (Overstreet et al., 1997).

<u>Effects of Pyridostigmine</u>

Pyridostigmine bromide is a quaternary carbamate anticholinesterase agent which has been used routinely in the treatment of myasthenia gravis. It was prescribed to Persian Gulf War participants as a prophylactic against the possible exposure to nerve agents. A subset of these individuals have reported very various problems, but it is not yet clear whether the problems are related to their exposure to pyridostigmine, to other agents during the Gulf War, or to stress. The present proposal addresses the hypothesis that the individuals developing these problems may have had a genetic cholinergic supersensitivity, undetectable under normal conditions, which made them more sensitive to pyridostigmine and/or other agents to which they were exposed. Because the FSL and FRL rats were genetically selected to respond differently to cholinergic agonists, they are ideal animals to test this hypothesis. It was predicted that the cholinergically supersensitive FSL rats would be more sensitive to the effects of pyridostigmine than the FRL rats or an outbred Sprague-Dawley strain of rats. The serum levels of growth hormone were selected as one variable to assess because there is evidence that pyridostigmine produces abnormal elevations of this hormone in several human populations with abnormalities (Chaudhury et al., 1997; Ghigo et al., 1993; Lucey et al., 1993; O'Keane et al., 1992, 1994). Telemetrically monitored core body temperature and general activity were selected as additional variables which could be measured reliably without influencing growth hormone levels and which might also be affected by pyridostigmine.

BODY

Methods

Animals. The FSL and FRL rats were selected from breeding colonies maintained at the University of North Carolina at Chapel Hill and randomly bred Sprague-Dawley (SD) rats (from which the FSL and FRL rats were originally derived) were obtained to act as a reference group. Both males

and females were used. The SD rats were included in the research design in order to determine whether both FSL and FRL rats are different from normal. They were maintained in groups of 3-5 in polypropylene cages under conditions of constant temperature and humidity and a reversed light:dark cycle (lights off from 1000-2200).

Surgery. Recording of locomotor activity and core body temperature in freely moving rats was accomplished by the implantation of a transmitter weighing 7.0 g (Model TA-11ETA-F40-L20). This transmitter has temperature- and motion-sensitive elements and when actuated by passing a magnet along the rat's abdomen, transmitted information to a computer where it was stored using Data Quest IV software (Data Sciences, Inc., St. Paul, MN).

At about 70 days of age the rats were injected i.p. with sodium pentobarbital (35 mg/kg) to induce anesthesia for implanting the telemetry transmitters, which provided continuous monitoring of core body temperature and general activity. The fur over the ventral abdominal area was clipped and a 3-cm longitudinal incision was made along the midline about 1 cm below the sternum. The radiotransmitter was inserted into the abdominal cavity and sutured to the peritoneal wall with 4-0 silk thread. After testing the transmitter with an AM receiver, the skin was closed. The rats were placed in single polypropylene cages after surgery and were closely monitored until they were active.

Procedures. After a one week period to allow full recovery (Rezvani et al., 1994), the FSL, FRL and SD rats were adapted to the home cages for at least 24 hr and then injected s.c. with a mixture of peripherally acting methyl atropine (MA, 2.0 mg/kg) and oxotremorine (OXO, 0.2 mg/kg) to determine hypothermic responses. This treatment was given to insure that each group of rats were either sensitive (FSL) or resistant (FRL) to a well characterized cholinergic agonist. This information is necessary to interpret the hypothermic responses to pyridostigmine.

Approximately three days after the MA/OXO challenge, the rats were given pyridostigmine (PYR) bromide by gavage. The design called for four groups (vehicle and 4, 12, 36 mg/kg), with ten rats per group. The animals were run in squads of 10 rats, the capacity of the computer, in a counterbalanced order. The average temperatures and general activity counts recorded during the hour preceding the gavage and those recorded at approximately 30 min after the injection were used in statistical analyses.

The rats were sacrificed by decapitation exactly 30 min after the oral administration of pyridostigmine, any signs of diarrhea were noted, and blood was collected into centrifuge tubes. The tubes were centrifuged and the plasma was collected and stored at -20 °C for later determination of growth hormone levels, using a kit obtained by NIDDK.

Results

Oxotremorine Challenge. As can be seen in Figure 1, the FSL rats exhibited a much more dramatic decrease in body temperature after the challenge with oxotremorine and methyl atropine than the FRL rats, as expected. However, it is also clear from this Figure that the randomly bred SD rats exhibit decreases in temperature that are intermediate between those of the FSL and FRL rats. Therefore, not only are the FSL rats more sensitive to this cholinergic challenge, but also the FRL rats are more resistant. These findings suggest that any effects of pyridostigmine in the lines should exhibit a similar pattern of differences if they are related to differences in cholinergic mechanisms.

Effects of Pyridostigmine. The effects of orally administered vehicle and pyridostigmine (PYR) on core temperature (upper panels) and general activity (lower panels) are illustrated in Figures 2-5. The baselines were the average scores for the one hour preceding oral administration and the treatment scores were those obtained at 30 min after the treatments, immediately prior to sacrifice.

Comparison of treatments with baselines indicated isolated instances of increases in activity (e.g., saline in SD females in Figure 2; 4 mg/kg PYR in FSL females in Figure 3), but there were no consistent line, sex, or treatment effects overall.

In contrast, there was a more consistent trend for the vehicle (Figure 2) and 4 and 12 mg/kg doses of PYR (Figures 3 and 4) to produce increases in core body temperature. To evaluate these changes, the baseline temperatures for each rat were subtracted from their temperatures 30 min after treatment and the means were calculated. These scores are summarized in Table 2. There were few line or sex differences in these scores, except for the 4 mg/kg dose of PYR, where the SD females exhibited higher temperatures than the other groups (Table 2). There was, however, a significant dose effect: The 36 mg/kg dose of PYR, unlike the vehicle or the lower doses, resulted in very small changes in core temperature (Figure 5, Table 2).

No consistent diarrhea was observed in any of the rats, so a table of these findings was not compiled. The blood samples are currently being tested for cholinesterase activity and growth hormone levels.

Discussion

These relatively small effects of PYR were not unexpected because it is a quaternary compound and does not normally get into the brain. However, Friedman et al. (1996) have shown that PYR can penetrate the blood-brain barrier in mice exposed to stressors, so it was thought that the FSL rats, which are more sensitive to stressors (See Overstreet, 1993; Overstreet et al., 1995), might exhibit a hypothermic response to PYR and the FRL rats would not. The fact that no consistent hypothermia was exhibited by any group strongly supports the conclusion that the blood-brain barrie is intact in these animals and that chollinergic agonists must be centrally active to produce decreases in body

temperature. Experiments on the effects of pyridostigmine in the two lines after exposure to stressors are needed to clarify this issue.

The higher dose of pyridostigmine, unlike the vehicle and the lower doses, did not lead to an elevation of core body temperature (Table 2). This finding could indicate that the high dose is having a hypothermic effect. However, because the effects were similar in all groups, we do not feel that these effects are based on cholinergic mechanisms, because there are substantial line differences in the hypothermic responses to oxotremorine. Because of the necessity to sacrifice the animals at 30 min, the peak time for elevation of growth hormone levels, it was not possible to examine the temperature and activity measures for longer periods of time. It is planned to conduct such experiments in the current year to elucidate the effects of the high dose of PYR on core body temperature.

As indicated above, the growth hormone assays are still in progress. We expect them to be quite revealing, because it has been well documented that PYR, despite its inability to penetrate the BBB, significantly increases growth hormone levels in both rats and humans (Martin et al., 1978; Mazza et al., 1994). In fact, patients with a variety of ailments, such as depression, obsessive compulsive disorders, and chronic fatigue syndrome, exhibit abnormally responses to PYR (Chaudhuri et al., 1997; Ghigo et al., 1993; Lucey et al., 1993; O'Keane et al., 1992, 1994). Since some of these patient groups exhibit behavioral symptoms overlapping with or similar to those described in Gulf War veterans, it is possible that they too may exhibit abnormal responses, but no such study is available as yet. The FSL and FRL rats may thus represent animal analogs of patient and control groups, respectively, and can be useful in elucidating the mechanism of action of PYR.

Table 1

Multiple Chemical Sensitivity in FSL Rats

Drug Classes to which FSL rats are more sensitive than FRL rats

Drug Class	Compound	Responses
Anticholinesterase	DFP	Temperature/drinking
Anticholinesterase	Physostigmine	Temperature/activity
Muscarinic Agonist	Oxotremorine	Temperature/activity
Muscarinic Agonist	Pilocarpine	Temperature/activity
Muscarinic Agonist	Arecoline	Temperature/activity
Nicotinic Agonist	Nicotine	Temperature/activity
Dopamine D1/2 Agonist	Apomorphine	Temperature
Dopamine D2 Agonist	Quinpirole	Temperature
Dopamine D2 Antagonist	Raclopride	Catalepsy
5-HT-1B Agonist	mCPP	Temperature/activity
5-HT-1A Agonist	8-OH-DPAT	Temperature
5-HT-1A Agonist	Buspirone	Temperature
Benzodiazepine Agonist	Diazepam	Temperature/activity
Multiple (GABA, 5-HT)	Ethanol	Temperature

Table 2

Change in Core Temperature after Oral Administration of

Saline or Pyridostigmine in FSL, FRL and SD Rats

Line/Sex	Dose of Pyridostigmine (mg/kg)			
	0.0	4.0	12.0	36.0
SD-male	+0.4±0.1	+0.6±0.1	+0.2±0.1	0.0±0.2
SD-female	+0.5±0.1	+1.0±0.1	+0.5±0.1	-0.2±0.2
FSL-male	+0.3±0.3	+0.3±0.2	+0.9±0.4	-0.2±0.2
FSL-female	+0.4±0.2	+0.7±0.1	+0.8±0.2	+0.1±0.2
FRL-male	+0.3±0.2	+0.2±0.1	+0.3±0.2	+0.1±0.2
FRL-female	+0.3±0.2	+0.4±0.2	+0.8±0.1	+0.2±0.1
One-Way ANOVA	0.50	5.17**	2.55	0.91

**Significant differences, $p < 0.01$

FIGURE LEGENDS

Figure 1. Hypothermic Effects of Oxotremorine in Telemetrically Monitored FSL, FRL and Sprague-Dawley (SD) Rats. Each point represents the mean temperatures over a 5-min interval for 10 males and 10 females in each group. A mixture of oxotremorine (0.2 mg/kg) and methyl atropine (2 mg/kg) was injected s.c. at the time indicated. Note that the FSL rats exhibit the greatest peak decreases in temperature and the Sprague-Dawley rats have intermediate responses.

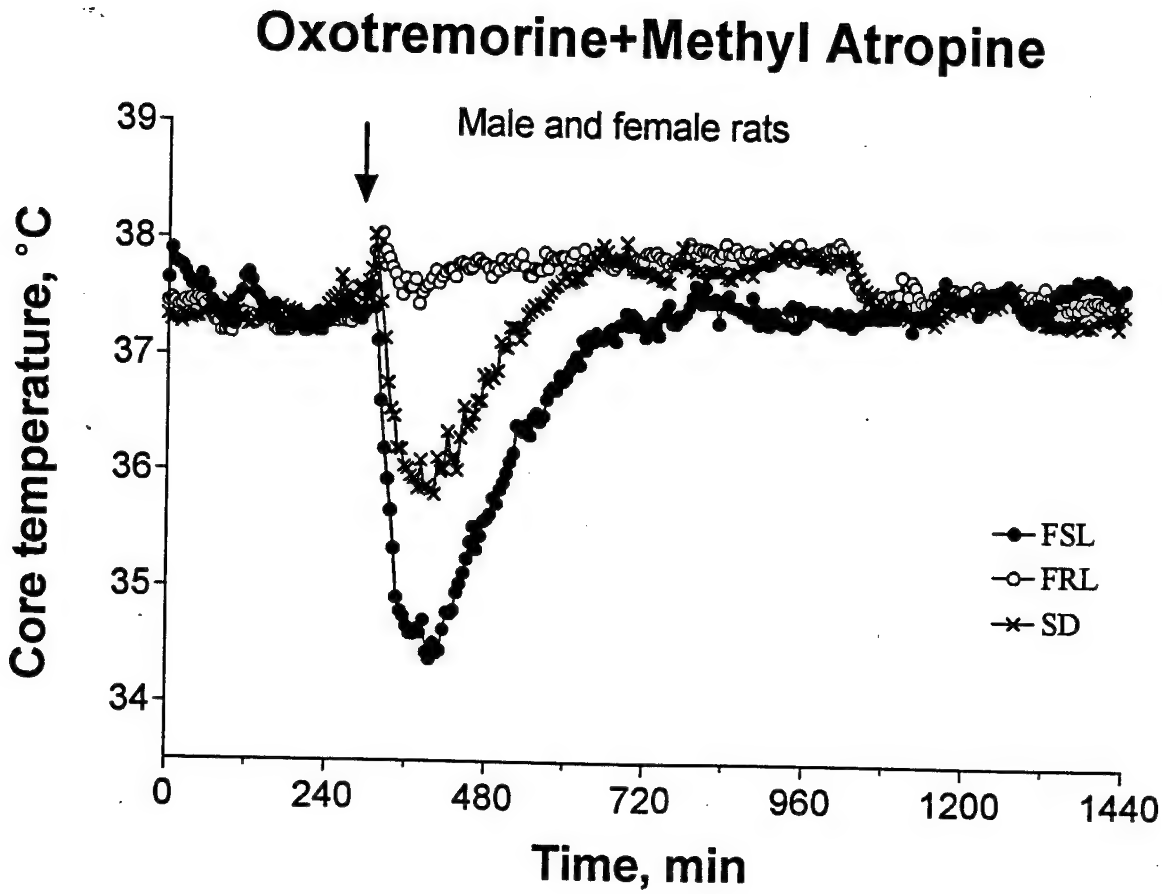
Figure 2. Effects of Orally Administered Saline Vehicle on Telemetrically Monitored Temperature (Upper panel) and General Activity (Lower panel) in FSL, FRL and SD Rats. Baselines are the averages over the one hour preceding the treatment; values for treatment are those recorded approximately 30 min after the treatment, immediately prior to sacrifice. *Significantly different, $p < 0.01$, from baseline according to related measures t tests.

Figure 3. Effects of 4 mg/kg Orally Administered Pyridostigmine (PYR) on Telemetrically Monitored Temperature (Upper panel) and General Activity (Lower panel) in FSL, FRL and SD Rats. Baselines are the averages over the one hour preceding the treatment; values for treatment are those recorded approximately 30 min after the treatment, immediately prior to sacrifice. *Significantly different, $p < 0.01$, from baseline according to related measures t tests.

Figure 4. Effects of 12 mg/kg Orally Administered Pyridostigmine (PYR) on Telemetrically Monitored Temperature (Upper panel) and General Activity (Lower panel) in FSL, FRL and SD Rats. Baselines are the averages over the one hour preceding the treatment; values for treatment are those recorded approximately 30 min after the treatment, immediately prior to sacrifice. *Significantly different, $p < 0.01$, from baseline according to related measures t tests.

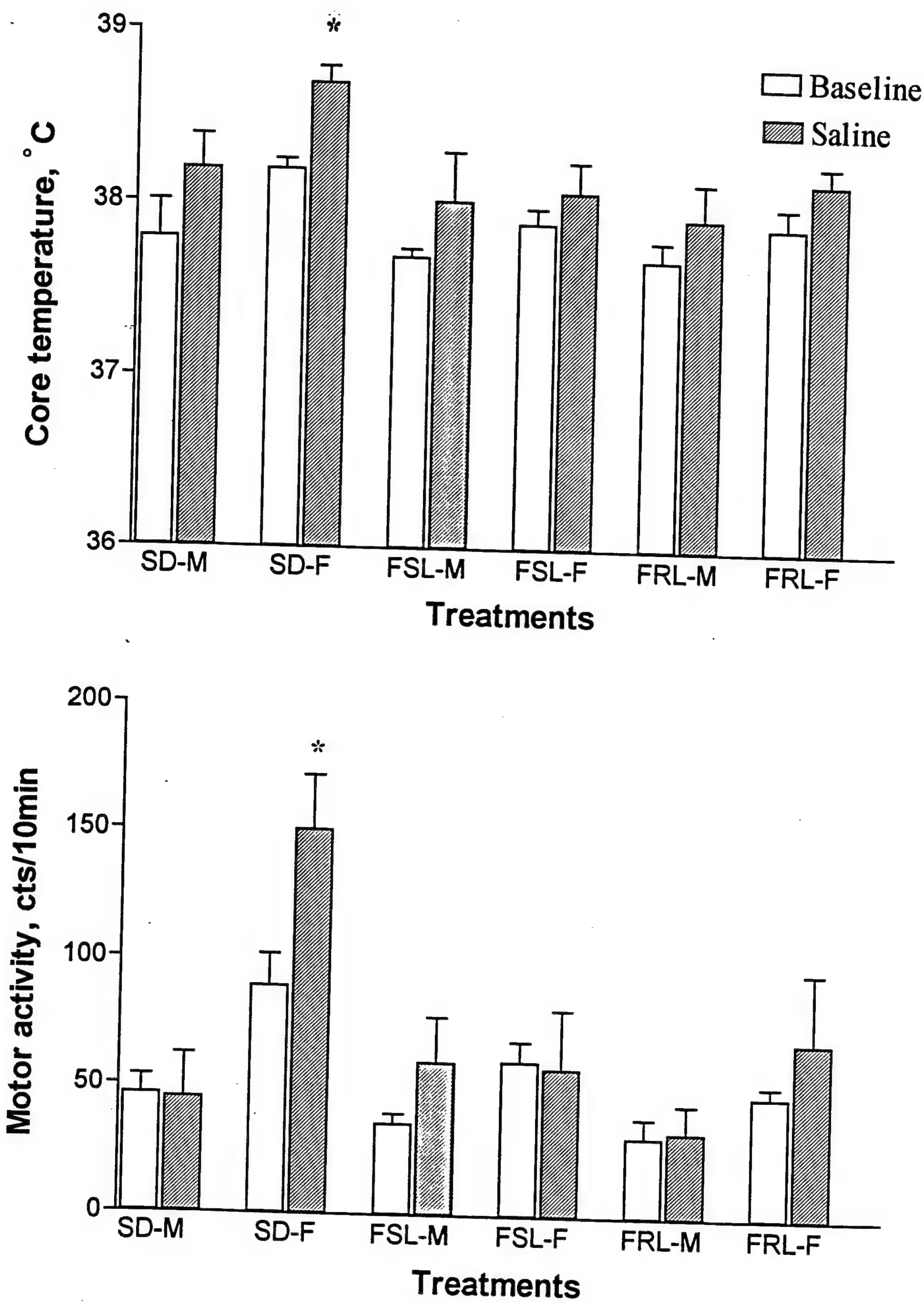
Figure 5. Effects of 36 mg/kg Orally Administered Pyridostigmine (PYR) on Telemetrically Monitored Temperature (Upper panel) and General Activity (Lower panel) in FSL, FRL and SD Rats. Baselines are the averages over the one hour preceding the treatment; values for treatment are those recorded approximately 30 min after the treatment, immediately prior to sacrifice. *Significantly different, $p < 0.01$, from baseline according to related measures t tests.

FIGURE 1



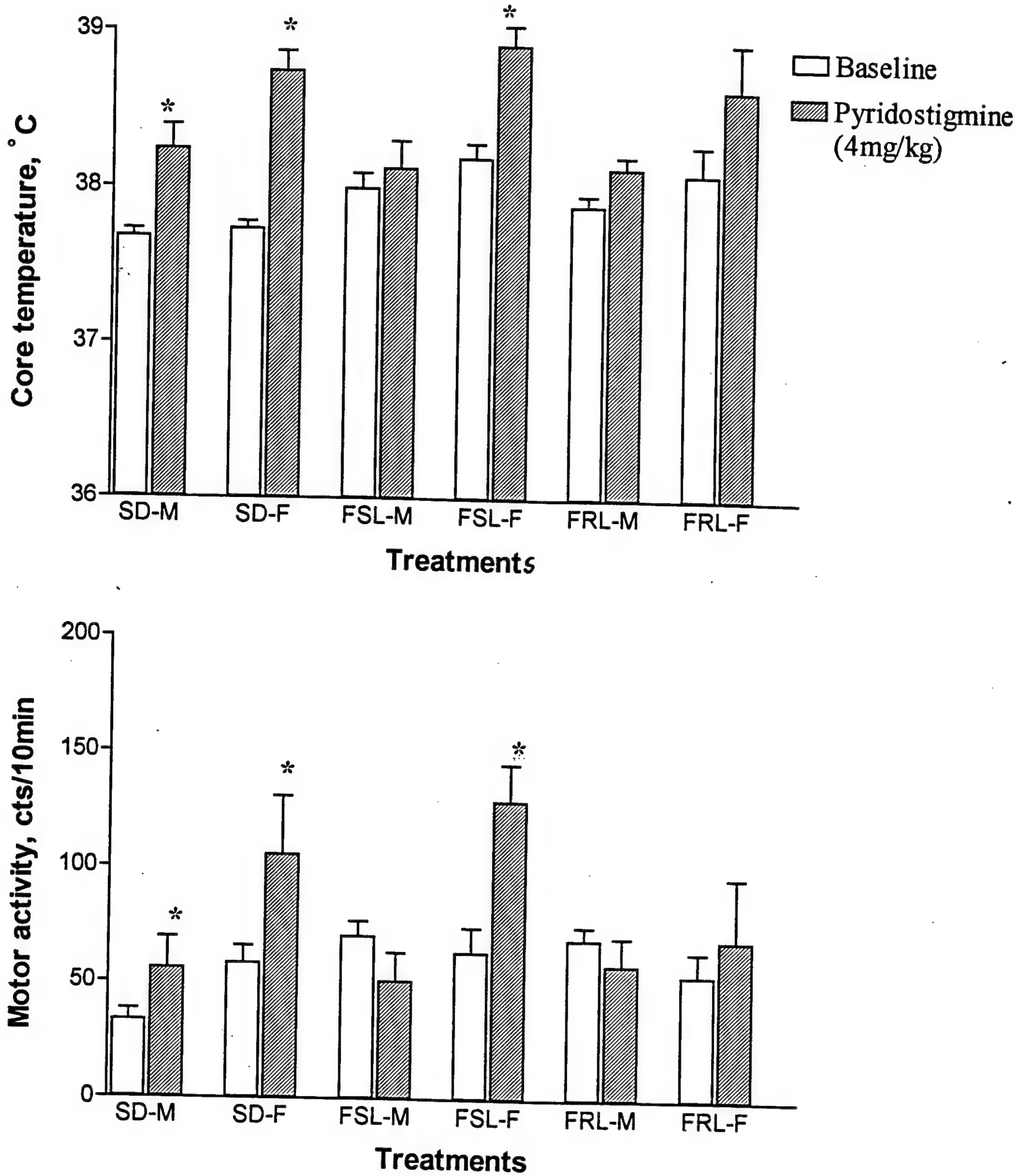
Oxotremorine+Methyl Atropine
Male and female rats
Core temperature, °C
39
38
37
36
35
34
0
240
480
720
960
1200
1440
Time, min
FSL
FRL
SD

FIGURE 2



39
38
37
36
*
Baseline
Saline
Core temperature, °C
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments
200
150
100
50
0
*
Motor activity, cts/10min
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments

FIGURE 3



Core temperature, °C
39
38
37
36
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments
Baseline
Pyridostigmine (4mg/kg)
Motor activity, cts/10min
200
150
100
50
0
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments

FIGURE 4

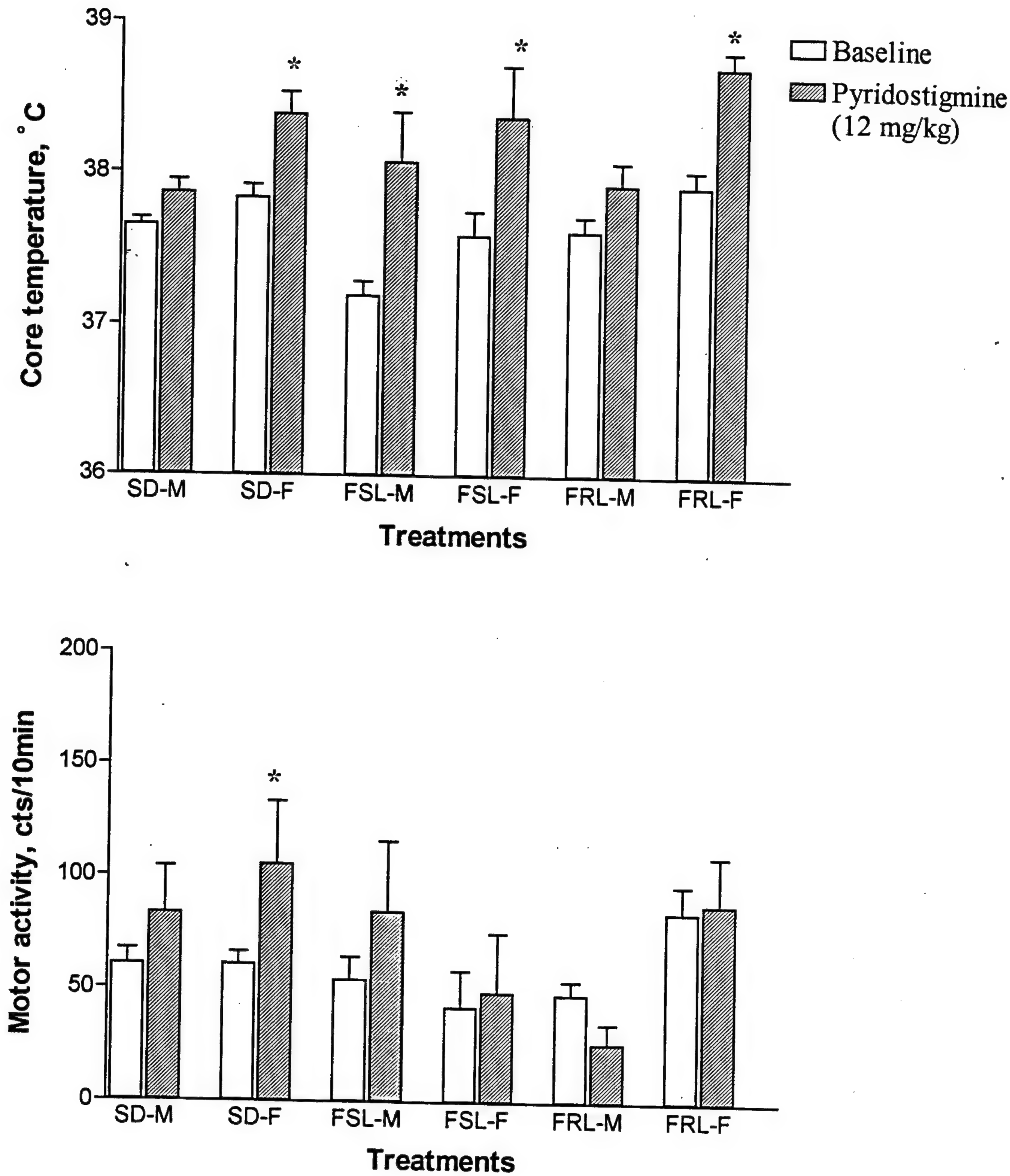
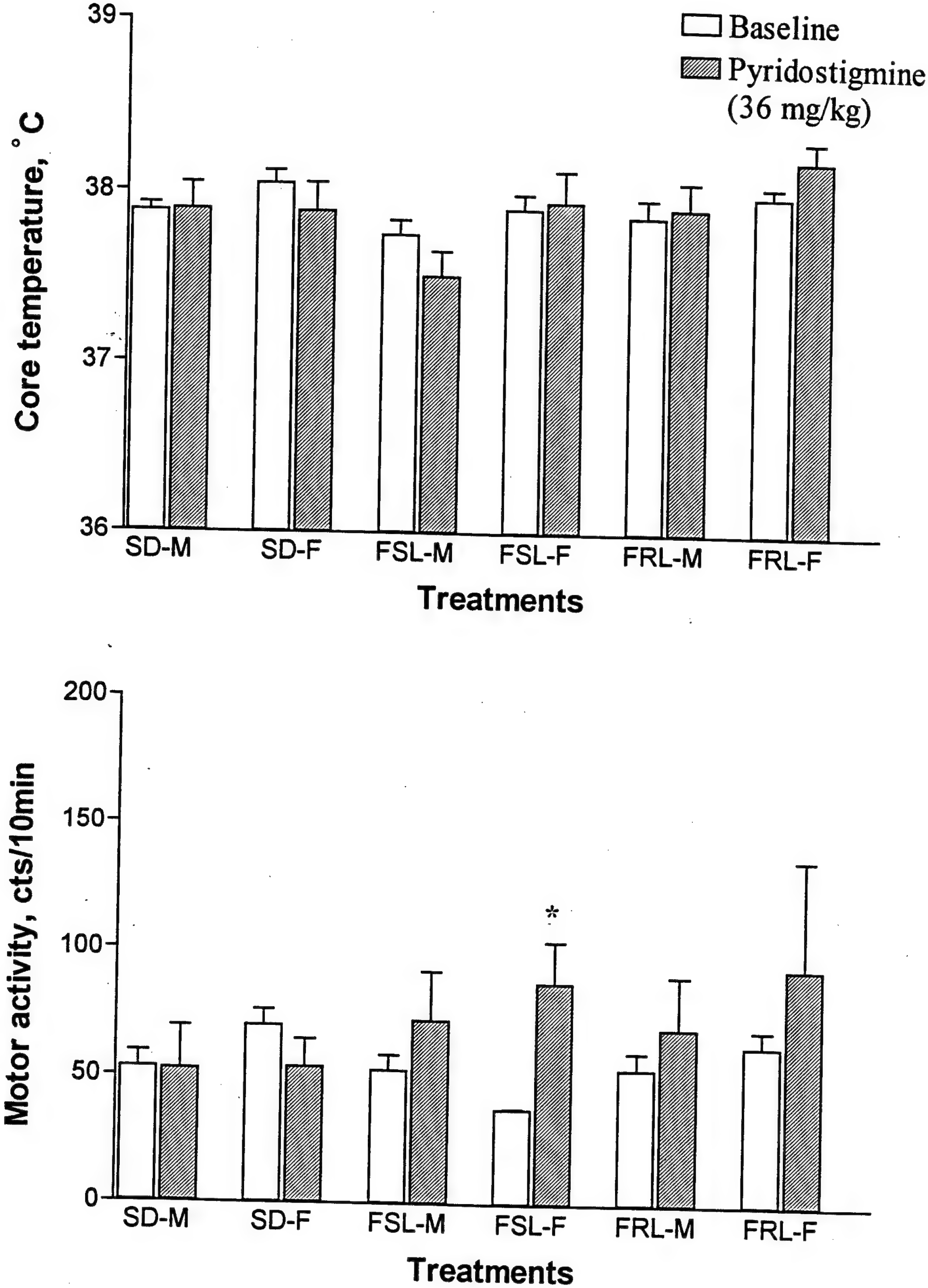

39
38
37
36
Core temperature, °C
*
*
*
*
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments
Baseline
Pyridostigmine
(12 mg/kg)
200
150
100
50
0
Motor activity, cts/10min
*
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments

FIGURE 5


Baseline
Pyridostigmine (36 mg/kg)
Core temperature, °C
39
38
37
36
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments
Motor activity, cts/10min
200
150
100
50
0
*
SD-M
SD-F
FSL-M
FSL-F
FRL-M
FRL-F
Treatments

CONCLUSIONS

The project encountered several difficulties which delayed the progress. Initial breeding difficulties with the FSL and FRL rats, which are estimated to be almost 90% inbred now, led to a delay in the start of the project. Initial studies with the telemetry equipment, which was originally purchased in 1993, indicated that the software was out-of-date and a new software package had to be ordered. Finally, our request for the growth hormone assay kit was lost in the system for several weeks. Because of these delays, we have not completed the growth hormone assays as yet and our conclusions about the first year's work must be incomplete. Each of these problems have been solved during the course of this past year, so we expect that the studies planned for 1997-1998 can proceed in a timely manner and that all of the work will be completed by the end of June, 1998.

PYR had relatively little effect on temperature and activity, as expected. Other work with cholinergic agents generally report that centrally active compounds must be given before changes in temperature and activity can be seen. The increases in these measures in the present study are probably related to the stress of handling to administer the compounds by gavage, rather than any pharmacological effects of the drug. This conclusion is based on the observation that increases were comparable after the vehicle and the low doses of PYR. The fact that there were no line differences in any of these responses is consistent with the above conclusion. Had PYR been producing cholinergically related effects, the FSL rats would be predicted to be more affected, as they were after oxotremorine.

It should be stressed that the "negative" results for pyridostigmine reported here were expected and will be useful comparative data for the growth hormone results, where differences are expected. The present report also includes positive data on the results for oxotremorine: They confirm that the FSL and FRL rats are both very different from the randomly bred SD rats (Figure 1).

REFERENCES

Arango, V., Ernsberger, P., Marzuk, P.M., Chen, J.S., Tierney, H., Stanley, M., Reis, D.J., and Mann, J.J. (1990) Autoradiographic demonstration of increased serotonin $5\text{-}HT_2$ and B-adrenergic receptor binding sites in the brain of suicide victims. Arch. Gen. Psychiatry 47, 1038-1047.

Arora, R.C. and Meltzer, H.Y. (1989) Serotonergic measures in the brains of suicide victims: $5\text{-}HT_2$ binding sites in the frontal cortex of suicide victims and control subjects. Am. J. Psychiatry 146, 730-736.

Ashford, N.A. and Miller, C.S. (1989) Chemical sensitivity. A report to the New Jersey State Department of Health.

Ashford, N.A. and Miller, C.S. (1991) Chemical Exposure: Low Levels and High Stakes, Van Nostrand Reinhold, New York.

Backheit, A.M., Behan, P.O., Dinan T.G., Gray, C.E., O'Keane, V. (1992) Possible upregulation of hypothalamus 5-Hydroxytryptamine receptors in patients with postviral fatigue syndrome. Brit. Med. J. 304, 1010-1012.

Bell, I.R., Miller, C.S., and Schwartz, G.E. (1992) An olfactory-limbic model of multiple chemical sensitivity syndrome: Possible relationships to kindling and affective spectrum disorders. Biol. Psychiatry 32, 218-242.

Benca, R.M., Obermeyer, W.H. Thisted, R.A., and Gillin, J.C. (1992) Sleep and psychiatric disorders: A meta-analysis. Arch. Gen. Psychiatry 49, 651-670.

Benca, R.M., Overstreet, D.H., Gilliland, M.A., Russell, D., Bergmann, B.M., Obermeycr, W.H. (1996) Increased basal REM dleep but no difference in dark induction or light suppression of REM sleep in Flinders Rats with cholinergic supersensitivity. Neuropsychopharmacology 15:45-51.

Black, D.W., Rathe, A., and Goldstein, R.B. (1990) Environmental illness. A controlled study of 26 subjects with "20th Century Disease". JAMA 264, 166-170.

Bushnell P.J., Levin, E.D., Overstreet, D.H. (1995) Spatial working and reference memory in rats bred for autonomic sensitivity to cholinergic stimulation: Acquisition, accuracy, speed, and effects of cholinergic drugs. Neurobiology of Learning and Memory 63, 116-132.

Chaudhuri A., Majeed T., Dinan T., Behan P.O. (1997) Chronic fatigue syndrome: A disorder of central cholinergic transmission. J. Chronic Fatigue 3, 3-16.

Cone, J.E. and Sult, T.A. (1992) Acquired intolerance to solvents following pesticide/solvent exposure in a building: a new group of workers at risk for multiple chemical sensitivities? Toxicol. Indust. Health 8, 29-39.

Cox, B., Kerwin, R.W., Lee, T.F., and Pycock, C.J. (1980) A dopamine-5-Hydroxytryptamine link in the hypothalamic pathways which mediate heat loss in the rat. J. Physiol. 303, 9-21.

Criswell, H.A., Overstreet, D.H., Rezvani, A.H., Johnson, K.B., Simson, P.E., Knapp, D.J., Moy, S.S., and Breese, G.R. (1994) Effects of ethanol, MK-801, and chlordiazepoxide on locomotor activity in different rat lines: Dissociation of locomotor stimulation from ethanol preference. Alcohol. Clin. Exp. Res. 18, 917-923.

Crocker A.D., and Overstreet, D.H. (1991) Changes in dopamine sensitivity in rats selectively bred for differences in cholinergic function. Pharmacol. Biochem. Behav. 38, 105-108.

Cullen, M.R. (1987) Workers with multiple chemical sensitivities. Occup. Med. State Art Rev. 2, 655-806.

Daws, L.C., Schiller, G.D., Overstreet, D.H., Orbach, J. (1991) Early development of muscarinic supersensitivity in a genetic animal model of depression. Neuropsychopharmacology 4, 207-217.

Fibiger, H.C., Lytle, L.D., and Campbell, B.A. (1970) Cholinergic modulation of adrenergic arousal in the developing rat. J. Comp. Physiol. Psychol. 3, 384-389.

Fiedler, N., Maccia, C., and Kipen, H. (1992) Evaluation of chemically sensitive patients. J. Occup. Med. 34, 529-538.

Friedman, A., Kaufer D., Shemer, J., Hendler, I., Soreq, H., Tur-Kaspar, I. (1996) Pyridostigmine brain penetration under stress enhances neronal excitability and induces immediate transcriptional response. Nature Med. 2, 1382-1385.

Gann, H., Riemann, D., Hohagen, F., Dressing, H., Muller, W.E., and Berger, M. (1992) The sleep structure of patients with anxiety disorders in comparison to that of healthy controls and depressive patients under baseline conditions and after cholinergic stimulation. J. Affect. Dis. 26, 179-190.

Ghigo, E., Nicolosi, M., Arvat, E., Marcone, A., Danelon, F., Mucci, M., Franceschi, M., Smirne, S., and Camanni, F. (1993) Growth hormone secretion in Alzheimer's disease: studies with growth hormone-releasing hormone alone and combined with pyridostigmine or arginine. Dementia 4, 315-320.

Gillin, J.C., Sutton, L., Ruiz, C., Kelsoe, J., Dupont, R.N., Darko, D., Risch, S.C., Golshan, S., and Janowsky, D. (1991) The cholinergic rapid eye movement induction test with arecolline in depression. Arch. Gen. Psychiatry 48, 264-270.

Janowsky, D.S. and Risch, S.C. (1987) Acetylcholine mechanisms in affective disorders. In: H.Y. Meltzer (Ed) Psychopharmacology. The Third Generation of Progress, Raven Press, New York, pp. 527-534.

Janowsky, D.S., Overstreet, D.H., and Nurnberger J.I.Jr. (1994) Is cholinergic sensitivity a genetic marker for the affective disorders? Am. J. Med. Genet. (Neuropsychiatric Genetics) 54, 335-344.

Klemm, W.R. (1989) Drug effects on active immobility responses: what they tell us about neurotransmitter systems and motor function. Prog. Neurobiol. 32, 403-422.

Lesch, K.P., Disselkamp-Tietze, J., and Schmidtke, A. (1990) 5-HT1A receptor function in depression: Effect of chronic amitriptyline treatment. J. Neural Transm. 80, 157-161.

Lucey, J.V., Butcher, G., Clare, A.W., and Dinan, T.G. (1993) Elevated growth hormone responses to pyridostigmine in obsessive-compulsive disorder: evidence of cholinergic supersensitivity. Am. J. Psychiatry 150, 961-962,

Martin, J.D., Durand, D., Gurd, W., Faille, G., Audet, J., and Brazeau, P. (1978) Neuropharmacological regulation of episodic growth hormone-releasing hormone release into hypophysial portal blood of conscious sheep. Endocrinology 133, 1247-1251.

Mazza, E., Ghigo, E., Boffano, G., Valeto, M., Naccarioi, M., Arvat, D., Bellone, J., Procopio, M., Muller, E.E., and Camanni, F. (1994) Effects of direct and indirect acetylcholine receptor agonists on growth hormone secretion in humans. Eur. J. Pharmacol. 254, 17-20.

Meltzer, H.Y., and Lowy, M.T. (1987) The serotonin hypothesis of depression. In Psychopharmacology: The Third Generation of Progress. In: H.Y. Meltzer (Ed.), Raven Press, New York, pp. 513-526.

Mikuni, M., Kusumi, I., Kagaya, A., Kuroda, Y., Mori, H., and Takahashi, K. (1991) Increased 5-HT-2 receptor function as measured by serotonin-stimulated phosphoinositide hydrolysis in platelets of depressed patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 15, 49-62.

Miller, C.S. (1994) White paper: Chemical sensitivity: history and phenomenology. Toxicol. Indust. Health 10, 253-276.

Miller, C.S. and Mitzel, H.C. (1995) Chemical sensitivity attributed to pesticide exposure versus remodeling. Arch. Env. Health (in press).

Nurnberger, J.I.Jr., Berrettini, W., Mendelson, W., Sack, D., and Gershon, E.S. (1989) Measuring cholinergic sensitivity: I. Arecoline effects in bipolar patients. Biol. Psychiatry 25, 610-617.

O'Keane, V., O'Flynn, K., Lucey, J., and Dinan, T.G. (1992) Pyridostigmine-induced growth hormone responses in healthy and depressed subjects - Evidence for cholinergic supersensitivity in depression. Psychol. Med. 22, 55-60.

O'Keane, V., Abel, K. and Murray, R.M. (1994) Growth hormone responses to pyridostigmine in schizophrenia: evidence for cholinergic dysfunction. Biol. Psychiatry 36, 582-586.

Overstreet, D.H. (1986) Selective breeding for increased cholinergic function: Development of a new animal model of depression. Biol. Psychiatry 21, 49-58.

Overstreet DH. (1989) Correlations of Ethanol-induced hypothermia in FSL and FRL rats with hypothermia induced by other drugs. Presented at 13th Annual Symposium of the North Carolina Alcoholism Research Authority, Raleigh.

Overstreet, D.H. (1993) The Flinders Sensitive Line rats: A genetic animal model of depression. Neurosci Biobehav Rev 17: 51-68.

Overstreet, D.H. and Janowsky, D.S. (1991) A cholinergic supersensitivity model of depression. In: A. Boulton, G. Baker, and M. Martin-Iverson, (Eds.) Neuromethods. Vol. 19: Animal Models in Psychiatry, II, Humana Press, Clifton, NJ, pp. 81-114.

Overstreet, D.H. and Measday, M. (1985) Impaired active avoidance performance in rats with cholinergic supersensitivity: Its reversal with chronic imipramine. Presented at 4th International Congress of Biological Psychiatry, Philadelphia, PA.

Overstreet, D.H. and Russell, R.W. (1982) Selective breeding for sensitivity to DFP. Effects of cholinergic agonists and antagonists. Psychopharmacology. 78, 150-154.

Overstreet D.H., Hadick, D.G., and Russell, R.W. (1972). Effects of amphetamine and pilocarpine on eating behavior in rats with chronically low acetylcholinesterase levels. Behav. Biol. 7, 212-226.

Overstreet, D.H., Kozar M.D., and Lynch, G.D. (1973) Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. Neuropharmacology. 12, 1017-1032.

Overstreet, D.H., Russell, R.W., Vasquez, B.J., and Dalglish, F.W. (1974) Involvement of muscarinic and nicotinic receptors in behavioral tolerance to DFP. Pharmacol. Biochem. Behav. 2, 45-54.

Overstreet, D.H., Russell, R.W., Helps, S.C., and Messenger, M. (1979) Selective breeding for sensitivity to the anticholinesterase, DFP. Psychopharmacology 65, 15-20.

Overstreet, D.H., Russell, R.W., Crocker, A.D., and Schiller, G.D. (1984) Selective breeding for differences in cholinergic function: Pre- and Post-synaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Research. 294, 327-332.

Overstreet, D.H., Booth, R., Dana, R., Risch, S.C., and Janowsky, D.S. (1986a) Enhanced elevation of corticosterone following arecoline administration to rats selectively bred for increased cholinergic function. Psychopharmacology 88, 129-130.

Overstreet, D.H., Janowsky, D.S., Gillin, J.C., Shiromani, P., and Sutin, E.L. (1986b) Stress-induced immobility in rats with cholinergic supersensitivity. Biol, Psychiatry. 21, 657-664.

Overstreet, D.H., Double, K., and Schiller, G.D. (1989a) Antidepressant effects of rolipram in a genetic animal model of depression: Cholinergic supersensitivity and weight gain. Pharmacol. Biochem. Behav. 34, 691-696.

Overstreet, D.H., Janowsky, D.H., and Rezvani, A.H. (1990a) Impaired active avoidance responding in rats selectively bred for increased cholinergic function. Physiol. Behav. 47, 787-788.

Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1990b) Increased hypothermic responses to ethanol in rats selectively bred for cholinergic supersensitivity. Alcohol & Alcohol., 25, 59-65.

Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1992a) Genetic animal models of depression and ethanol preference provide support for cholinergic and serotonergic involvement in depression and alcoholism. Biol. Psychiatry 31, 919-936.

Overstreet, D.H., Russell, R.W., Hay, D.A., and Crocker, A.D. (1992b) Selective breeding for increased cholinergic function: Biometrical genetic analysis of muscarinic responses. Neuropsychopharmacology 7, 197-204.

Overstreet, D.H., Janowsky, D.S., Pucilowski, O., and Rezvani, A.H. (1991) Swim test immobility cosegregates with serotonergic but not cholinergic sensitivity in cross breeds of Flinders Line rats. Psychiat. Genet. 4, 101-107.

Overstreet, D.H., Pucilowski, O., Rezvani A.H., and Jaowsky, D.S., (1995) Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. Psychopharmacology 121, 27-37.

Overstreet D.H., Miller, C.M., Janowsky, D.S., Russell, R.W. (1996) A potential animal model of multiple chemical sensitivity with cholinergic supersensitivity. Toxicology 111, 119-134.

Overstreet, D.H., Rezvani, A.H., Yang Y., Hamedi H., Janowsky, D.S. (1997) Animal model of chemical sensitivity involving cholinergic agents. Presented at Toxicology in Risk Assessment Symposium held in Bethesda, MD, May 14-16, 1997.

Pepe, S., Overstreet, D.H., and Crocker, A.D. (1988) Enhanced benzodiazepine responsiveness in rats with increased cholinergic function. Pharmacol. Biochem. Behav. 31, 15-20.

Pucilowski, O. (1987) Monoaminergic control of affective aggression. Acta Neurobiol. Exp. 47, 25-50.

Pucilowski, O. and Overstreet, D.H. (1993) Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. Pharmacol. Biochem. Behav. 32, 471-475.

Pucilowski, O., Eichelman, B.S., Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1991b) Enhanced affective aggression in genetically bred hypercholinergic rats. Neuropsychobiology. 24, 37-41.

Pucilowski, O., Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1993). Chronic mild stress-induced anhedonia: Greater effect in a genetic rat model of depression. Physiol. Behav. 54, 1215-1220.

Ray, A., Sen, P., and Alkondon, M. (1989) Biochemical and pharmacological evidence for central cholinergic regulation of shock-induced aggression. Pharmacol. Biochem. Behav. 32, 867-871.

Rezvani, A.H., Overstreet, D.H., Ejantkar, A., and Gordon, C.J. (1994) Autonomic and behavioral responses of selectively bred hypercholinergic rats to oxotremorine and diisopropyl fluorophosphate. Pharmacol. Biochem. Behav. 48, 703-707.

Rosenthal, N. and Cameron, C.L. (1991) Exaggerated sensitivity to an organophosphate pesticide (letter). Am. J. Psychiatry 148, 270.

Russell, R.W. and Overstreet, D.H. (1987) Mechanisms underlying sensitivity to organophosphorus anticholinesterase agents. Prog. Neurobiol. 28, 97-129.

Russell, R.W., Overstreet, D.H., Messenger, M., and Helps, S.C. Selective breeding for sensitivity to DFP. Generalization of effects beyond criterion variables. Pharmacol. Biochem. Behav. 17:885-891, 1982.

Schiller, G.D., and Overstreet, D.H. (1993) Selective breeding for increased cholinergic function: Preliminary study of nicotinic mechanisms. Medic. Chem. Res. 2, 578-583.

Schiller, G.D., Orbach, J., and Overstreet, D.H. (1988) Effects of intracerebroventricular administration of site selective muscarinic drugs in rats genetically selected for differing cholinergic sensitivity. Presented at meeting of Australasian Society for Clinical and Experimental Pharmacology, Adelaide, December.

Schiller, G.D., Daws, L.C., Overstreet, D.H., Orbach, J. (1991) Absence of anxiety in an animal model of depression with cholinergic supersensitivity. Brain Res. Bull. 26, 443-447.

Schiller, G.D., Pucilowski, O., Wienicke, C., and Overstreet, D.H. (1992) Immobility-reducing effect of antidepressants in a genetic animal model of depression. Brain Res. Bull. 28, 821-823.

Schreiber, W., Lauer, C.J., Krumrey, K., Holsboer, F., and Krieg, J.C. (1992) Cholinergic REM sleep induction test in subjects at high risk for psychiatric disorders. Biol. Psychiatry 32, 79-90.

Shiromani, P.J., Overstreet, D.H., Levy, D., Goodrich, C.A., Campbell, S.S., and Gillin, J.C. (1988) Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropsychopharmacology 1, 127-133

Sihotang, K. and Overstreet, D.H. (1983) Studies on the possible relationship of brain proteins to behavioral sensitivity to DFP. Life Sci. 32, 413-420.

Simon, G.E., Katon, W.J., and Sparks, P.J. (1990) Allergic to life: Psychological factors in environmental illness. Am. J. Psychiatry 147, 901-906.

Sitaram, N., Jones, D., Dube, S., Keshavan, M., Bell, J., Davies, A., and Reynal, P. (1987) The association of supersensitive cholinergic-REM induction and affective illness within pedigrees. J. Psychiatry Res. 21, 487-497.

Wallis, E., Overstreet, D.H., and Crocker, A.D. (1988) Selective breeding for increased cholinergic function: Increased serotonergic sensitivity. Pharmacol. Biochem. Behav. 31, 345-350.

APPENDIX - Animal Model of Chemical Sensitivity Involving Cholinergic Agents

* David H. Overstreet, Amir H. Rezvani, Ying Yang, Mani Hamedi, Davis S. Janowsky

Abstract

Risk assessment procedures need to take into account the possibility of individual differences in drug sensitivity. To illustrate this point this paper will summarize data collected on the Flinders Line rats, which are differentially sensitive to a variety of chemical agents, including cholinergic agonists. The Flinders Line rats were developed at Flinders University in Australia by selective breeding for differential responses to the anticholinesterase, diisopropyl fluorophosphate (DFP). Separation of two lines, the Flinders Sensitive Line (FSL) and the Flinders Resistant Line (FRL), was apparent by 8 generations, with the FSL rats being more sensitive to the hypothermic effects of DFP. Subsequently, it was determined that the FSL rats were also more sensitive to directly acting muscarinic agonists, such as oxotremorine and pilocarpine. This increased sensitivity to DFP and muscarinic agonists might be related to the muscarinic receptor elevations seen in the hippocampus, striatum, and hypothalamus of the FSL rats. Because increased sensitivity to muscarinic agonists in the FSL rats is comparable to that seen in depressed humans, various behavioral tests were conducted and the data from these were consistent with the hypothesis that the FSL rats may be a genetic animal model of depression: they are less active in a novel open field, have lower appetites and body weights, are more sensitive to stressors, and their behavioral immobility is ameliorated by chronic treatment with antidepressants. The FSL rats have also been determined to be more sensitive to the effects of a variety of other drugs, including alcohol, diazepam, nicotine, and 8-OH-DPAT, a 5-HT1A receptor agonist. This increased sensitivity to a variety of drugs in FSL rats is reminiscent of human patients suffering from multiple chemical sensitivity (MCS) and suggests that MCS might arise, in part, from genetically influenced muscarinic supersensitivity. The heightened sensitivity of the FSL rats to a variety of drugs suggests that they will also be more sensitive to the effects of pyridostigmine, an anticholinesterase which was given to gulf war participants. The results of initial experiments indicate that there are no differences in hypothermia after pyridostigmine, but FSL rats may be more sensitive to the bradycardia induced by pyridostigmine.

Key Words: Animal Model of MCS; Organophosphate DFP; FSL Rats; Human Depressives; Cholinergic Supersensitivity; Gulf War Illness

Introduction

In the assessment of risk to individuals exposed to known or potential toxicological agents. there needs to be a consideration of the possibility that especially sensitive populations exist. For example, some individuals have reported side effects after taking pyridostigmine to protect them against potential nerve gas exposure and others have not. Other individuals have reported increased sensitivity to a variety of chemical agents, usually after a triggering exposure to a specific chemical such as an organophosphate pesticide (e.g., Miller and Mitzel, 1995). The hypothesis that a genetically based cholinergic supersensitivity might underlie the increased sensitivity of these vulnerable human populations will be addressed in the present communication by describing in detail the features of an animal model with cholinergic supersensitivity which is also more sensitive to a variety of drugs and other chemical agents and which may, therefore, mimic the human condition labelled Multiple Chemical Sensitivity (MCS). In the final section of this paper some initial results on the effects of pyridostigmine on this animal model will be presented.

The validity of an animal model rests in part on its similarity in structure and function to a target condition in humans. The closer the similarity to the human condition the model is, the greater is the probability that manipulations of one will provide information valid for extrapolation to the other. A final test of validity comes when predictions made from the animal model and applied to the human condition are shown to be accurate. To evaluate the model proposed below, it is important to summarize the observed clinical characteristics of MCS.

MULTIPLE CHEMICAL SENSITIVITY

Multiple Chemical Sensitivity (MCS) is a syndrome in which, following acute or repeated exposure to one or more chemicals, most commonly organophosphate pesticides (OPs), individuals become overly sensitive to a wide variety of chemically-unrelated compounds. These can include ethanol, caffeine and other psychotropic drugs (Ashford and Miller, 1989, 1991; Bell et al., 1992; Cullen, 1987; Miller, 1994). The symptoms of MCS often reported include fatigue, cognitive difficulties, depression, irritability, headaches, dyspnea, digestive problems, musculoskeletal pain, and

numbness in their extremities. These conditions often overlap those of common medical illnesses such as depression, somatization disorder, chronic fatigue syndrome, fibromyalgia, asthma and others. However, a distinguishing feature of MCS is the strong belief of the patients that their symptoms are brought on by common exposures to low levels of volatile organic chemicals such as fragrances, insecticides, traffic exhaust, disinfectants and perfumes.

Descriptions of MCS have been noted in various journals for more than 40 years. In recent years, occupational medicine physicians in universities have reported seeing increasing numbers of individuals who appear to have it. In addition, there have been three federally-sponsored workshops focussed on MCS (Association of Occupational and Environmental Clinics, 1992; National Research Council, 1992; Mitchell and Price, 1994). Sponsoring agencies have included The National Research Council (NRC), the Agency for Toxic Substances and Disease Registry (ATSDR), the Environmental Protection Agency, and the National Institute of Environmental Health Sciences (NIEHS). The recommendations from these meetings have repeatedly stressed the need for further research on the condition and the development of animal models.

MCS has been described as a two-step process that is analogs to but different from the process that occurs in allergic diseases (Ashford and Miller, 1991): For both allergies and MCS there is *Induction* (initiation, sensitization or loss of tolerance) as a consequence of an initial chemical exposure or to sensitization to bee venom, for example. In both conditions, there is also subsequent *triggering* of symptoms; however, in MCS this may occur from exposure to a wide range of chemically-diverse substances, while in allergy antibodies are highly specific and spreading of sensitivities to chemically unrelated substances does not occur.

MCS patients most frequently report their condition as being induced by pesticides, especially OPs and carbamates (Ashford and Miller, 1991; Miller and Mitzel, 1995). Significantly, exposures to OP and carbamate agents during the Gulf War included pesticides, pyridostigmine bromide (used as a prophylaxis for nerve agents), and, possibly, low levels of actual nerve agents. Although chemicals in this class can inhibit cholinesterase, rarely have cholinesterase levels been measured in sporadic MCS cases, and frequently symptoms typically associated with cholinesterase inhibition are absent among

individuals who report ultimately developing MCS as a consequence of OP exposure. While acute OP toxicity has generally been considered to be reversible, provided it is not fatal, the toxicology literature contains a variety of examples of individuals who were exposed to these agents and later showed persistent psychological, psychiatric, or neuropsychological deficits (Gershon and Shaw, 1961; Rosenstock et al., 1991; Rowntree at al., 1950; Savage et al., 1988; Tabershaw and Cooper, 1966). To account for these long-lasting effects it has been proposed that OPs may damage cholinergic receptors or in other ways induce injury independent of their ability to inhibit cholinesterase (Gupta and Abou-Donia, 1994; Huff et al., 1994).

Several case reports of individuals developing MCS after exposure to pesticides (Rosenthal and Cameron, 1991; Cone and Sult, 1992) have appeared recently. Even more recently, Miller and Mitzel (1995) surveyed 112 MCS patients, 37 of whom attributed their illness to exposure to an OP or carbamate pesticide and the other 75 to remodelling of a building a procedure which commonly involves exposures to low levels of mixed solvents emanating from fresh paint, carpeting, glues, etc. Following their initial exposure, both groups reported similar symptoms and similar intolerances to chemicals, foods, ethanol, and caffeine. However, overall, the pesticide-exposed group reported significantly greater symptom severity. The authors interpreted these findings as suggesting a possible common pathway for the development of MCS, despite the fact that the two groups initially experienced exposures to very different classes of chemicals. They hypothesized that the relatively greater neurotoxicity and/or potency of the cholinesterase inhibitors as compared to mixed low-level solvents might account for the greater symptom severity in the pesticide-exposed individuals.

An important observation in this field is that MCS patients usually report that other individuals simultaneously exposed to similar amounts of pesticides, e.g, family members, friends, or co-workers, did not develop MCS or even experience transient illness. This observation suggests that a subset or subsets of the people may be more vulnerable to developing MCS. Indeed, some (Black et al., 1990; Simon et al., 1990), but not all (Fiedler et al., 1992) researchers have reported greater rates of depression and somatization disorder predating the "initiating" chemical exposure among persons with

MCS as compared to controls. Thus, any model must take into account why only some individuals develop MCS after exposures to pesticides or other chemicals.

One such model which will be described in the subsequent sections of this paper is the FSL (Flinders Sensitive Line) rat. This rat was developed by selective breeding for increased sensitivity to an OP, so it shares some etiological similarity to patients with MCS who were exposed to pesticides.

AN ANIMAL MODEL

The FSL rat model is one with which we have had extensive experience, particularly in research on depressive syndromes (Overstreet, 1993; Overstreet and Janowsky, 1991; Overstreet et al., 1995). Analogies between depressed states and MCS, as well as substance hypersensitivities in FSL rats, first brought our attention to the potential value of this model for experimental studies of MCS, as recently described (Overstreet et al., 1996). Further, because the FSL rats were selectively bred for increased responses to the organophosphate, DFP, it is possible that they may have some special relevance to Gulf War Illness, commonly reported in individuals exposed to the carbamate, pyridostigmine. Some preliminary findings of our work with pyridostigmine will be presented in the final section of this paper.

Selective Breeding for OP Differences

The FSL rat model arose from a selective breeding program designed to produce two lines of rats, one with high (FSL) and one with low (Flinders Resistant Line - FRL) sensitivity to the anticholinesterase agent, diisopropylfluorophosphate (DFP) (Overstreet et al., 1979; Russell et al., 1982). The selective breeding program, which was initiated at Flinders University in Adelaide, Australia, utilized three somatic measures of DFP (Overstreet et al., 1979; Russell et al., 1982). A rank-order system was used to give equal weighting to each of the three variables. Rats which had the lowest average ranks were intermated to establish and maintain the line of more sensitive rats (FSL), while rats which had the highest average ranks were intermated to establish and maintain the line of more resistant rats (FRL). Subsequent studies showed that randomly bred Sprague-Dawley rats, from which the lines were originally derived, were not different from the FRL rats. On the other hand, FSL

rats were significantly more sensitive to DFP than the other two groups (Overstreet et al., 1979; Russell et al., 1982).

Biochemical Mechanisms

This project was initiated, in part, to develop genetically resistant lines of rats so that the biochemical mechanisms of resistance could be compared with those of tolerance. Early studies ruled out changes in acetylcholinesterase as a mechanism to account for the differential sensitivity of FSL and FRL rats to DFP (Overstreet et al., 1979; Russell and Overstreet, 1987; Sihotang and Overstreet, 1983), just as has been found for tolerance development (See Russell and Overstreet, 1987). Because DFP-tolerant rats were subsensitive to the effects of muscarinic agonists (e.g., Overstreet et al., 1973, 1974), the effects of muscarinic agonists on the FSL and FRL rats were examined (Overstreet 1986; Overstreet and Russell, 1982; Overstreet et al., 1986a,b). These studies showed that the FSL rats were more sensitive to pilocarpine, arecoline and oxotremorine than were the FRL rats; this supersensitivity was seen for a variety of responses, including hypothermia, reduced locomotor activity, and suppression of bar-pressing for water reward (Overstreet and Russell, 1982). Thus, FSL rats, developed by selectively breeding for increased sensitivity to DFP, exhibited opposite changes in sensitivity to muscarinic agonists compared to DFP-tolerant rats.

Biochemical studies indicated that the FSL rats exhibited greater numbers of muscarinic receptor binding sites in the hippocampus and striatum than the FRL rats (Overstreet et al., 1984; Pepe et al., 1988)., but there were no differences in acetylcholine turnover (Overstreet et al., 1984). Thus, once again, the FSL rats appear to represent the converse of DFP-tolerant rats; having increased numbers of receptors rather than reduced numbers (See Russell and Overstreet, 1987). It appears that both tolerance and acute sensitivity to cholinergic agents is related to postsynaptic cholinergic mechanisms rather than presynaptic. Although in both instances, there have been detectable changes in the muscarinic receptors themselves, there are some findings, such as the increased sensitivity of FSL rats to noncholinergic agents (See Section below), which suggest that post-receptor mechanisms may also contribute.

Behavioral Features of FSL Rats

The FSL and FRL rats differ on a large number of behavioral tasks, as recently summarized in several review papers (Overstreet et al., 1995, 1996). In this section we will highlight a number of the key differences. The FSL rats have been reported to have lower locomotor activity than the FRL rats under a number of experimental conditions (Bushnell et al., 1995; Overstreet, 1986; Overstreet and Russell, 1982) but not all (Criswell et al., 1994; Rezvani et al., 1994). They are even less active when stressed prior to exposure to the open field (Overstreet, 1986; Overstreet et al., 1989a).

Results from several other behavioral paradigms are consistent with the view that depressive-like psychomotor retardation symptoms are more apparent in the FSL rats after exposure to stressors. For example, the FSL rats are impaired in active avoidance paradigms compared to the FRL rats (Overstreet and Measday, 1985; Overstreet et al., 1990a, 1992a). Another stress-oriented paradigm which has provided important information about behavioral differences between FSL and FRL rats is the forced swim test. Upon initial exposure in a cylinder (18-20 cm diameter) of water (25 ^{o}C), FSL rats are more immobile than the FRL rats (Overstreet, 1986; Overstreet et al., 1986a, Pucilowski and Overstreet, 1993; Schiller et al., 1992). This exaggerated immobility of the FSL rats is counteracted by chronic but not acute treatment with antidepressants (Overstreet, 1993; Pucilowski and Overstreet, 1993; Schiller et al., 1992). These findings provide further support for the contention that the FSL rat is a useful animal model of depression.

There are also differences in reward-related behaviors between the FSL and FRL rats which are consistent with the proposal that the FSL rats are a model of depression. In operant bar-pressing tasks, the FSL rats bar-pressed at lower rates and had to be maintained at a lower percentage of their free-feeding body weight and have smaller food pellets (37 vs. 45 mg) in order to keep their motivation sufficiently high to complete the session (Bushnell et al., 1995; Overstreet and Russell, 1982). Despite these differences in reward-related and stress-related behaviors, there appears to be no differences between the FSL and FRL rats in the ability to perform a matching-to-sample task (Bushnell et al., 1995). However, this test was carried out under normal, unstressed conditions, and it is not clear whether similar findings would obtain under stressed conditions. For example, FSL and FRL rats have

similar amounts of saccharin consumption under baseline conditions, but the FSL rats exhibit greater decreases after exposure to chronic mild stress (Pucilowski et al., 1993).

The FSL rats also have elevated REM sleep and reduced latency to REM sleep (Shiromani et al., 1988, Benca et al., 1996), as has been reported in human depressives (Benca et al., 1992) Human depressives are also more sensitive to the effects of cholinergic agonists on REM sleep latency (Janowsky et al., 1994), but there are no data in the FSL rats regarding drug effects on sleep.

In sum, the FSL rats and depressed humans exhibit a large number of behavioral and physiological similarities (See Overstreet, 1993; Overstreet et al., 1995, 1996, for more detailed accounts).

Multiple Chemical Sensitivity in FSL Rats

Clinical observations suggest that MCS may be initiated by acute or chronic exposure to a variety of chemical agents (Miller and Mitzel, 1995). Because the FSL rats were selectively bred to have increased responses to the anticholinesterase agent, DFP, it should not be surprising that they exhibited increased sensitivity to muscarinic agonists (Daws et al., 1991; Overstreet, 1986; Overstreet and Russell, 1982; Overstreet et al., 1992a,b; Schiller et al., 1988). It has also been reported that human depressives are also more sensitive to directly acting muscarinic agonists (Gann et al., 1992; Gillin et al., 1991) as well as anticholinesterases (Gann et al., 1992; Janowsky and Risch, 1987; Nurnberger et al., 1989; O'Keane et al., 1992; Schreiber et al., 1992; Sitaram et al., 1987). A similar increased sensitivity to anticholinesterases has been observed in MCS patients (Cone and Sult, 1992; Miller and Mitzel, 1995; Rosenthal and Cameron, 1991), but there are no published data for MCS patients regarding sensitivity to direct cholinergic agonists. FSL rats are also more sensitive to nicotine, which interacts with nicotinic cholinergic receptors (Schiller and Overstreet, 1993).

The cholinergic system interacts with many other major neurotransmitter systems, including serotonergic, dopaminergic, GABAergic, and noradrenergic. Having animals with clear-cut differences in the cholinergic system afforded us the opportunity to test how the FSL and FRL rats differ in response to drugs interacting with these other neurotransmitter systems. Evidence from various drug challenge studies, in which relatively selective drugs are given to FSL and FRL rats, have revealed a

substantial number of differences between the FSL and FRL rats, as summarized in Table 1. FSL rats were found to exhibit a greater degree of hypothermia after a variety of drugs which interact with the serotonin 5-HT1A receptor (Wallis et al., 1988; Overstreet et al., 1992a, 1994). This outcome is consistent with much of the evidence suggesting supersensitive serotonergic mechanisms in depressives (Arango et al., 1990; Arora and Meltzer, 1989; Mikuni et al., 1991), but is not consistent with neuroendocrine studies reporting blunted responses to serotonergic agonists, which suggests serotonergic hyposensitivity (Lesch et al., 1990; Meltzer and Lowy, 1987). There are no data on the effects of selective serotonergic agents in MCS patients, but there is one report of supersensitive responses in individuals with chronic fatigue syndrome, which is related to MCS (Backheit, et al., 1992).

To date no evidence has been obtained to indicate any differences in responses to noradrenergic agents in the FSL rats (Overstreet, 1989; Overstreet et al, 1989a). In contrast, there are quite a number of differences with regard to dopaminergic agents (Table 1). The FSL rats are supersensitive to the hypothermic (Crocker and Overstreet, 1991) and aggression-promoting (Pucilowski et al., 1991a) effects of apomorphine, a mixed D1/D2 agonist, and quinpirole, a selective D2 agonist. On the other hand, the FSL rats were subsensitive to the stereotypy-inducing effects of similar doses of the same compounds and there were no apparent differences in dopamine D2 receptors between FSL and FRL rats (Crocker and Overstreet, 1991). These opposite changes in sensitivity in the various functions might be related to the type of modulation of these functions by the cholinergic and dopaminergic systems. Stimulation of both cholinergic and dopaminergic systems promotes hypothermic and aggressive responses (Cox et al., 1980; Pucilowski, 1987; Ray et al., 1989), but cholinergic stimulation reduces activity and stereotypy, thereby opposing the effects of dopaminergic stimulation (Fibiger et al., 1970; Klemm, 1989).

The FSL and FRL rats are differentially sensitive to the effects of several pharmacological agents which have modulatory roles at the GABA-A receptor, as summarized in Table 1. However, as with the case of dopamine agonists, the differential effects are observed only for some actions of the drugs, not for all. For example, the hypothermic effects of ethanol are significant higher in the FSL rats compared to the FRL rats, but the sedative effects are similar (Overstreet et al., 1990b). Similarly, the

behavioral suppressant effects of diazepam are significantly greater in the FSL rats (Pepe et al., 1988), but its anxiolytic effects in the two lines are comparable (Schiller et al., 1991). The fact that these two commonly abused psychotropic drugs both modulate GABA function at the GABA-A receptor suggests that there might be differences in GABA-A receptor subtype composition between the two lines, but there is not biochemical evidence for such differences as yet. Furthermore, despite differences in sensitivity to the hypothermic effects of ethanol, the FSL and FRL rats do not differ in their rates of voluntary ethanol consumption (Overstreet et al., 1992a).

In summary, it appears that the FSL rat is more sensitive to a variety of chemical agents in addition to the OP anticholinesterase agent for which they were selectively bred. In this regard, the FSL rat is somewhat analogous to MCS patients who have become more sensitive to a range of agents following exposure to OP anticholinesterases. The extent of the similarity between the FSL rats and MCS patients, on one hand, and human depressives and MCS patients, on the other, will be further evaluated in the next section.

FSL RATS RESEMBLE MCS AND DEPRESSED PATIENTS

As Table 2 summarizes, the behavioral features of individuals with MCS and those of depressed patients and FSL rats are strikingly similar in regard to weight, appetite, activity and stressability, hedonia, and sleep. There are also some uncertainties in Table 2, suggesting several studies that might be carried out in MCS patients to test further the extent of the associations among the three groups. For example, polysomnographic recordings of sleep in asymptomatic MCS patients would be particularly informative, especially since there is evidence that the REM sleep changes seen in depressed patients may be a trait marker of this disorder (Benca et al., 1992; Janowsky et al., 1994). Since REM sleep alterations can also be related to altered cholinergic mechanisms in general (Shiromani et al., 1987; Janowsky et al., 1994), a finding of REM sleep changes in MCS patients would suggest that altered cholinergic mechanisms might underlie abnormal sensitivity to chemicals. Such a finding would also be consistent with a cholinergic hypothesis as one possible explanation for the similarity between the MCS patients and depressives.

Another similarity between MCS and depressed patients is the ratio of females to males affected: There are many more females than males expressing the symptoms (Table 2). In general, twice as many females than males report depressive symptoms (Goodwin and Jamison, 1990). Similarly, the ratio of female to male MCS patients reaches 4/1 in some studies (Miller and Mitzel, 1995). Again, there is some parallel between the rats and humans because adult female FSL rats are more sensitive to cholinergic agonists than their male counterparts (Netherton and Overstreet, 1983). The possible greater sensitivity of adult females to cholinergic agonists might therefore partially account for the greater incidence of depression (Overstreet et al., 1988) and MCS in women.

Given the behavioral similarities between MCS patients and those who are depressed (Table 2), it is likely that depressed patients might be hypersensitive to similar drugs. Unfortunately, as described in Table 3, there is very little information about the sensitivity of depressed individuals to the range of drugs reported to cause problems in MCS patients, other than depressives' supersensitivity to anticholinesterases and cholinergic agonists (Janowsky et al., 1994). There is somewhat more evidence for a general increase in sensitivity to drugs in the FSL rats (Tables 1 & 3). It is particularly noteworthy that the FSL rats are more sensitive to both alcohol (Overstreet et al., 1990b) and nicotine (Schiller and Overstreet, 1993). The information on the effects of alcohol and nicotine in depressed patients is more complex, as implied by the question mark in Table 3. There are many studies reporting an interaction of depression with primary alcoholism on one hand (e.g., Kendler et al., 1993; Maier et al., 1994; Schuckit, 1986) and an interaction of smoking with depression on the other (Breslau et al., 1991; Glassman, 1993). Indeed, smoking cessation leads to depression in remitted depressives (Glassman, 1993). However, we are not aware of any studies specifically stating that depressed patients report intolerances for alcohol and/or nicotine.

It should be stressed that FSL rats may also be less sensitive to certain drugs (Crocker and Overstreet, 1991; Pucilowski et al., 1991). Furthermore, depressed patients exhibit blunted hormonal responses to a number of drugs affecting serotonergic and noradrenergic systems (Meltzer and Lowy, 1987). Consequently, more data from depressed individuals and FSL rats must be collected on their sensitivities to a broader range of chemicals. If the cholinergic system supersensitivity is one mechanism

underlying MCS, depression and the FSL rats, then it would be predicted that both FSL rats and depressed individuals would be more sensitive to such drugs. What is also needed are additional data on depressed individuals and FSL rats with respect to the triggering of symptoms by chemical or food exposures (Table 3).

Although we have emphasized the strong possibility of a cholinergic link between MCS patients, depressed patients, and FSL rats, other neurotransmitter systems may be involved. Serotonin has been implicated in depression (Meltzer and Lowy, 1987) and recent experiments on the Flinders rats suggest that serotonergic mechanisms may play an important role in some of their altered behaviors (Overstreet et al., 1994). However, there are no data on serotonergic mechanisms in MCS patients.

A somewhat more complex neurotransmitter model proposes that the various neurochemical systems interact with one another and that abnormal behavioral states may arise from an alteration in one system which creates an imbalance in its interactions with others. For example Janowsky et al. (1972) proposed that depression and mania were the consequence of imbalances between the noradrenergic and cholinergic systems, with depression being associated with relative cholinergic overactivity and mania being associated with relative noradrenergic overactivity. An animal parallel to this observations was reported by Fibiger et al. (1970). This model can account for some of the effects observed in the FSL rats following administration of noncholinergic drugs. For example, FSL rats are more sensitive to the hypothermic effects of dopamine agonists, but less sensitive to their sterotypy-inducing effects (Table 1; Crocker and Overstreet, 1991). Since dopaminergic and cholinergic systems work in parallel to regulate temperature but in opposition to regulate activity and stereotypy, an overactive cholinergic system could account for the findings with the dopamine agonists (See Overstreet, 1993). A similar argument could be made for chollinergic-serotonergic interactions as underlying depression and MCS.

Another type of mechanism which could underlie all MCS, depression and FSL rats is a change in second messenger rather than neurotransmitter functions. Several investigators have proposed that changes in G proteins, cyclic AMP or other second messenger systems may be involved in depression (Lesch and Manji, 1992; Avissar and Schreiber, 1992; Wachtel, 1989). Furthermore, it has been argued

that the functional muscarinic responses in the FSL and FRL rats are too divergent to be accounted for by the relatively small differences noted in muscarinic receptors (Overstreet, 1993). This "downstream" hypothesis may more easily account for the pervasiveness of the chemical sensitivity described in MCS patients, which involves many classes of chemical compounds besides those having direct effects on neurotransmitter systems. Differences in second messengers could be hereditary or induced by exposure to chemical agents or by the effects of chemical agents on cholinergic or monoaminergic mechanisms. Further study of FSL rats, MCS patients, and depressed patients using diverse approaches is needed to obtain a greater understanding of the mechanisms that may underlie MCS.

PRELIMINARY FINDINGS ON PYRIDOSTIGMINE

We propose that the characteristics of the animal model we have described are sufficiently analogous to MCS to warrant its use in testing hypotheses about the etiology and mechanisms of action involved in the syndrome. An example of the type of experimental protocols suggested by this review is the study of FSL and FRL rats after exposure to volatile solvents and other chemicals to which MCS patients report intolerance. This could be done with or without pre-existing exposure to cholinergic agents. If FSL rats do exhibit increased sensitivity to a wide variety of chemical agents, then treatment approaches could be attempted, for example using antidepressant drugs. It should be emphasized that proposing antidepressant treatment does not presume that depression is the cause of MCS; indeed, quite the reverse might be true. For example, exposure to OPs might augment cholinergic sensitivity, leading to both MCS and depression. The possibility that increased cholinergic sensitivity might underlie both MCS and depression suggests further experiments in these patient groups. Questions which could be explored are whether there is a subset of depressed patients who report intolerance to varied substances and whether these same patients exhibit a greater sensitivity to cholinergic agents? A further question could be whether this subset of depressed patients would benefit from avoidance of certain drugs and environmental exposures. Finally, it would be of interest to know whether MCS patients have altered cholinergic responsivity, particularly in light of a recent study demonstrated that chronic fatigue

syndrome, which is related to MCS, is associated with cholinergic supersensitivity (Chaudhuri et al., 1997).

Another research direction that could be taken is to propose that individual differences in cholinergic sensitivity may have, in part, accounted for the varied responses of Gulf War participants to pyridostigmine and other agents. Given the large differences in cholinergic sensitivity between the FSL and FRL rats, we would predict substantial differences in responses to pyridostigmine in these animals. The remainder of this section will summarize the preliminary results of our findings.

FSL and FRL rats were selected from breeding colonies maintained at the University of North Carolina at Chapel Hill and randomly bred Sprague-Dawley (SD) rats (from which the FSL and FRL rats were originally derived) were obtained to act as a reference group. Both males and females were used. At about 70 days of age the rats were injected i.p. with sodium pentobarbital (35 mg/kg) to induce anesthesia for implanting the telemetry transmitters, which provided continuous monitoring of core body temperature, general activity, and, in some cases, heart rate.

After a one week period to allow recovery, the FSL, FRL and SD rats were adapted to the home cages for 24 hr and then injected s.c. with a mixture of peripherally acting methyl atropine (MA, 2.0 mg/kg) and oxotremorine (OXO, 0.2 mg/kg) to determine hypothermic responses. As can be seen in Figure 1, the FSL rats exhibited the greatest hypothermic responses to OXO, as expected. However, the randomly bred SD rats were significantly more hypothermic than the FRL rats, suggesting that both lines have now diverged from control rats.

Approximately three days after the MA/OXO challenge, the rats were given pyridostigmine (PYR) bromide by gavage. The design called for four groups (vehicle and 4, 12, 36 mg/kg), but only the initial results from the two higher doses will be reported here. Temperature and activity were continuously recorded for 30 min after the PYR. The rats were then sacrificed by decapitation and blood removed and stored for the later analysis for cholinesterase activity and growth hormone levels. These assays are still in progress, so we will report on the physiological results in this communication; in addition, because the effects of PYR were not very striking, with little or no evidence of line differences, only the FSL and FRL data will be presented.

PYR had relatively few detectable effects on core body temperature at 12 mg/kg (Fig. 2A, 2B). There appeared to be a line difference in the females at 36 mg/kg (Fig. 3A), but neither the FSL nor the FRL rats exhibited any obvious hypothermia. In contrast, both male FSL and FRL rats exhibited modest but similar hypothermic responses to 36 mg/kg PYR (Fig. 3B). There were no detectable line differences in the effects of PYR on activity (data not shown).

These relatively small effects of PYR were not unexpected because it is a quaternary compound and does not normally get into the brain. However, Friedman et al. (1996) have shown that PYR can penetrate the blood-brain barrier in mice exposed to stressors, so it was thought that the FSL rats, which are more sensitive to stressors (See Overstreet, 1993; Overstreet et al., 1995), might exhibit a hypothermic response to PYR and the FRL rats would not. The fact that both male groups exhibit very similar small responses after 36 mg/kg PYR suggests that they both have intact blood-brain barriers. Experiments on the effects of pyridostigmine in the two lines after exposure to stressors are needed to clarify this issue.

As indicated above, the growth hormone assays are still in progress. We expect them to be quite revealing, because it has been well documented that PYR, despite its inability to penetrate the BBB, significantly increases growth hormone levels in both rats and humans (Martin et al., 1978; Mazza et al., 1994). In fact, patients with a variety of ailments, such as depression, obsessive compulsive disorders, and chronic fatigue syndrome, exhibit abnormally responses to PYR (Chaudhuri et al., 1997; Ghigo et al., 1993; Lucey et al., 1993; O'Keane et al., 1992, 1994). Since some of these patient groups exhibit behavioral symptoms overlapping with or similar to those described in Gulf War veterans, it is possible that they too may exhibit abnormal responses, but no such study is available at yet. The FSL and FRL rats may thus represent animal analogs of patient and control groups, respectively, and can be useful in elucidating the mechanism of action of PYR.

Acknowledgements

The work described in this paper has been supported in part by funding provided by the Australian Research Grants Committee, the National Health and Medical Research Committee of Australia, and the U.S. Army. We express our appreciation to Dawn Forte and Elijah Clark, Jr., for technical support.

Table 1

Multiple Chemical Sensitivity in FSL Rats

A. Drug Classes to which FSL rats are more sensitive than FRL rats

Drug Class	Compound	Responses
Anticholinesterase	DFP	Temperature/drinking
Anticholinesterase	Physostigmine	Temperature/activity
Muscarinic Agonist	Oxotremorine	Temperature/activity
Muscarinic Agonist	Pilocarpine	Temperature/activity
Muscarinic Agonist	Arecoline	Temperature/activity
Nicotinic Agonist	Nicotine	Temperature/activity
Dopamine D1/2 Agonist	Apomorphine	Temperature
Dopamine D2 Agonist	Quinpirole	Temperature
Dopamine D2 Antagonist	Raclopride	Catalepsy
5-HT-1B Agonist	mCPP	Temperature/activity
5-HT-1A Agonist	8-OH-DPAT	Temperature
5-HT-1A Agonist	Buspirone	Temperature
Benzodiazepine Agonist	Diazepam	Temperature/activity
Multiple (GABA, 5-HT)	Ethanol	Temperature

Table 2

Comparison of Characteristics and Behavioral Features of

MCS Patients, FSL Rats and Depressed Patients

MEASURE	MCS PATIENTS	FSL RATS	DEPRESSED PATIENTS
Weight	up or down	down	up or down
Appetite	up or down	down	up or down
Blood Pressure	up or down	ND	up or down
Food Craving	++	+	+
Sleep Disturbances	+++	++	+++
Loss of Drive	+++	+++	+++
Reduced Activity	+++	+++	+++
Cognitive Disturbance	+++	+/-	+++
Gender Ratios (F/M)	4/1	F>M	2/1

ND = Not Determined

Table 3

Comparison of Drug Sensitivity in MCS Patients, FSL Rats and Depressed Patients

COMPOUND	MCS PATIENTS	FSL RATS	DEPRESSED PATIENTS
Anticholinesterases	+++	+++	+++
Solvents, etc.	+++	ND	ND
Ethanol	+++	++	+?
Nicotine	+++	++	+?
Xanthines	+++	ND	ND
Foods	+++	ND	ND

ND = Not Determined.

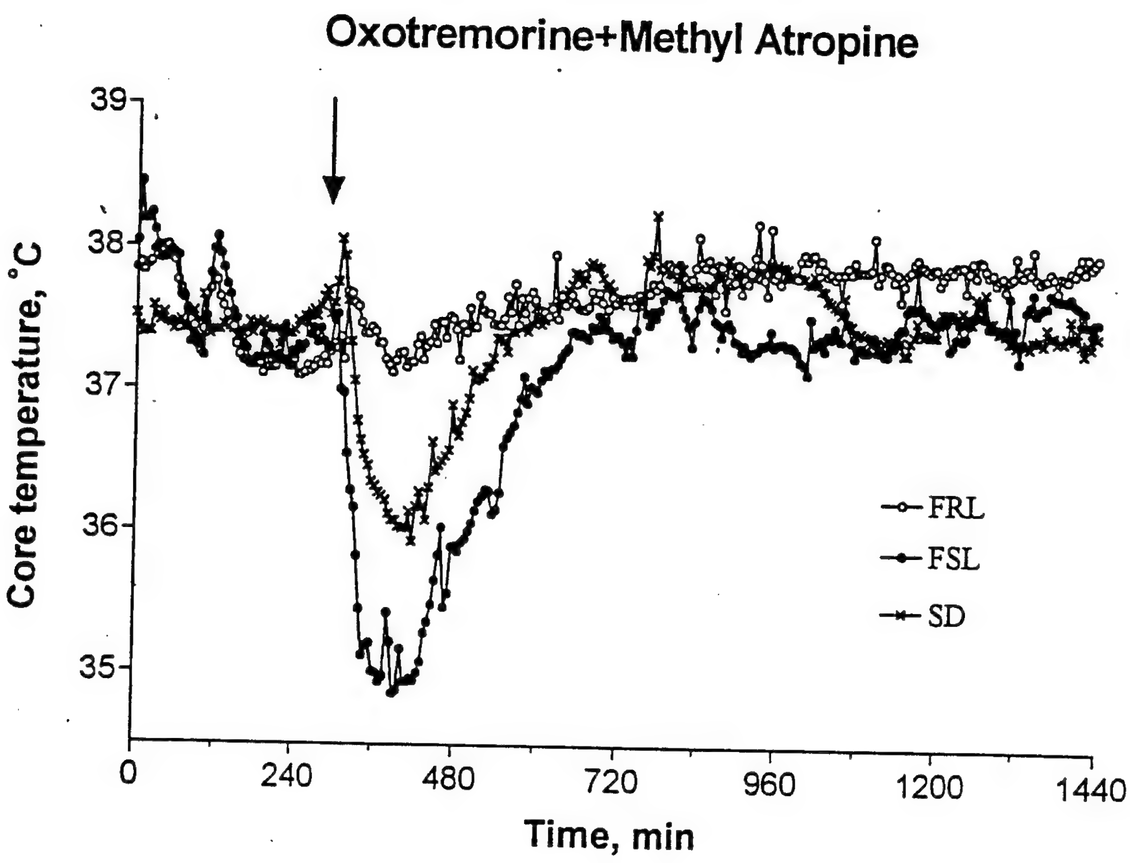
FIGURE CAPTIONS

FIGURE 1. Hypothermic Effects of Oxotremorine in Telemetrically Monitored FSL, FRL and Sprague-Dawley rats. The results are the mean temperatures of 10 males and 10 females in each group. Note that the FSL rats exhibit the greatest peak decreases in temperature and the Sprague-Dawley rats have intermediate responses.

FIGURE 2. The Effects of Pyridostigmine (12 mg/kg, orally) on Core Body Temperature in Male (A) and Female (B) FSL and FRL rats. The results are the mean temperatures of 5 animals per group.

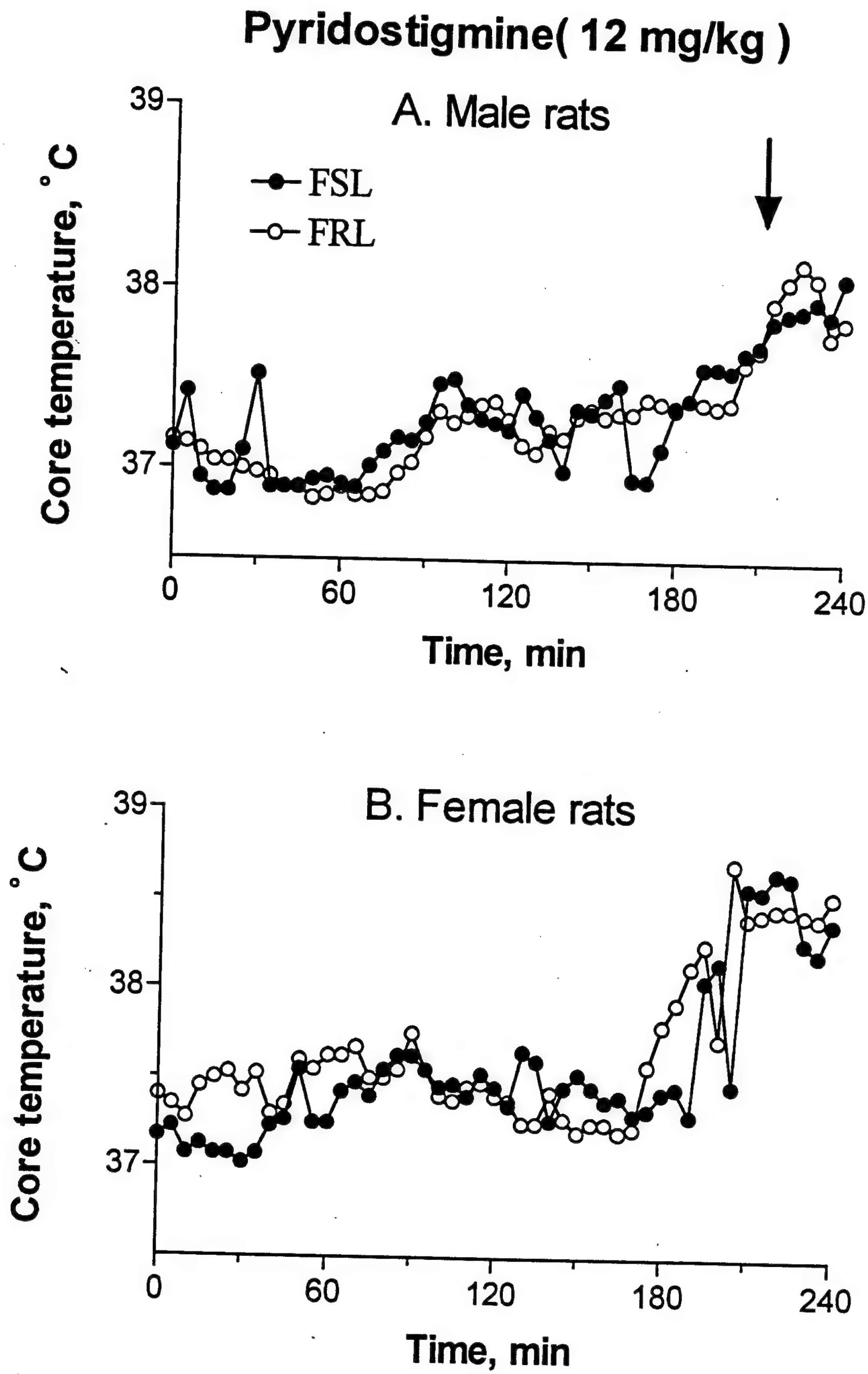
FIGURE 3. The Effects of Pyridostigmine (36 mg/kg, orally) on Core Body Temperature in Male (A) and Female (B) FSL and FRL rats. The results are the mean temperatures of 5 animals per group.

FIGURE 1



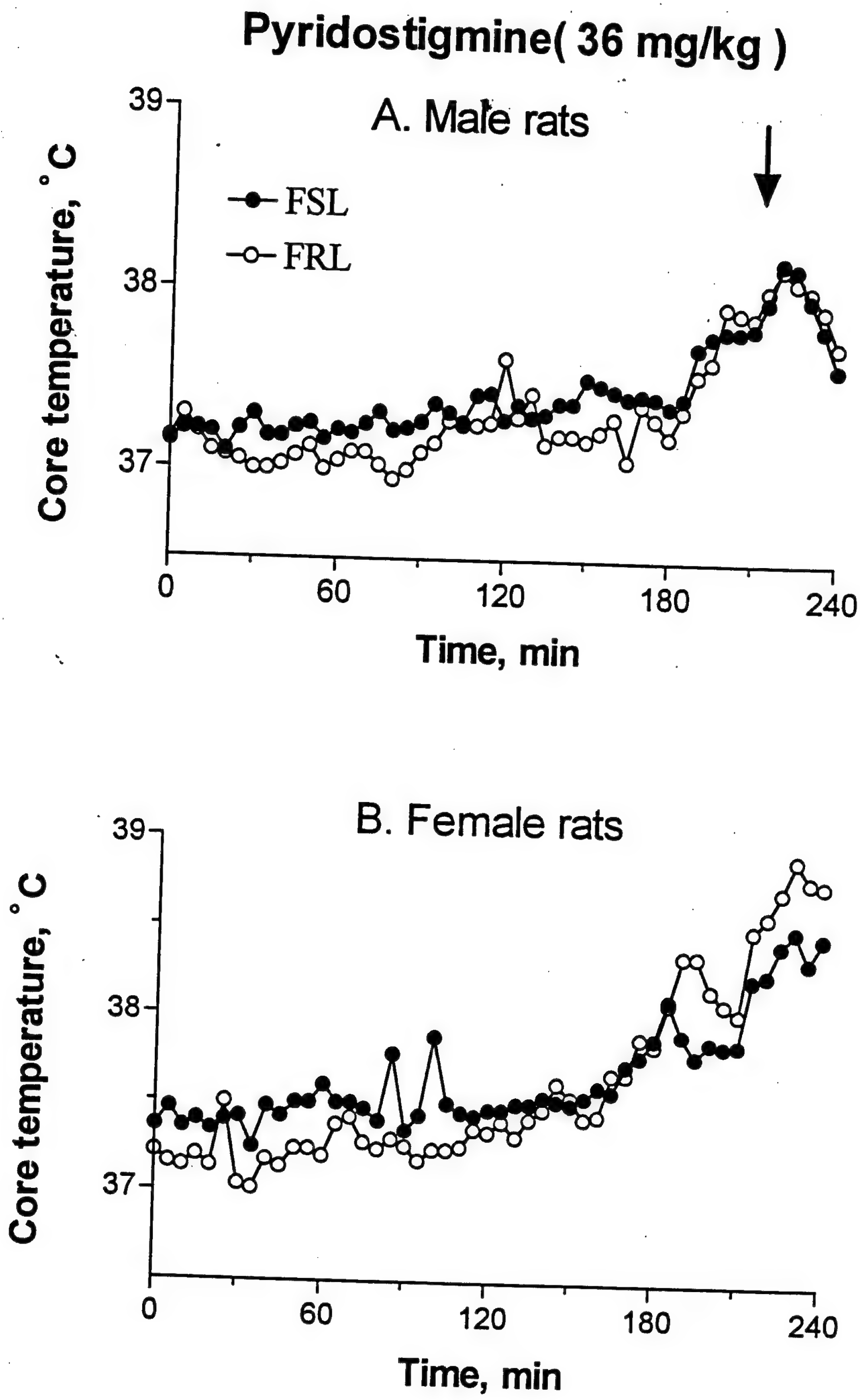
Oxotremorine+Methyl Atropine
39
38
37
36
35
Core temperature, °C
0
240
480
720
960
1200
1440
Time, min
FRL
FSL
SD

FIGURE 2



Pyridostigmine(12 mg/kg)
A. Male rats
FSL
FRL
Core temperature, °C
39
38
37
0
60
120
180
240
Time, min
B. Female rats
Core temperature, °C
39
38
37
0
60
120
180
240
Time, min

FIGURE 3



Pyridostigmine(36 mg/kg)
A. Male rats
FSL
FRL
Core temperature, °C
39
38
37
0
60
120
180
240
Time, min
B. Female rats
Core temperature, °C
39
38
37
0
60
120
180
240
Time, min

REFERENCES

Arango, V., Ernsberger, P., Marzuk, P.M., Chen, J.S., Tierney, H., Stanley, M., Reis, D.J., and Mann, J.J. (1990) Autoradiographic demonstration of increased serotonin 5-HT_2 and B-adrenergic receptor binding sites in the brain of suicide victims. Arch. Gen. Psychiatry 47, 1038-1047.

Arora, R.C. and Meltzer, H.Y. (1989) Serotonergic measures in the brains of suicide victims: 5-HT_2 binding sites in the frontal cortex of suicide victims and control subjects. Am. J. Psychiatry 146, 730-736.

Ashford, N.A. and Miller, C.S. (1989) Chemical sensitivity. A report to the New Jersey State Department of Health.

Ashford, N.A. and Miller, C.S. (1991) Chemical Exposure: Low Levels and High Stakes, Van Nostrand Reinhold, New York.

Association of Occupational and Environmental Clinics (1992) Advancing the understanding of multiple chemical sensitivity. Toxicol. Indust. Health 8, 1-257.

Avissar, S., and Schreiber, G. (1989) Muscarinic receptor subclassification and G-proteins: Significance for lithium action in affective disorders and for the treatment of extrapyramidal side effects of neuroleptics. Biol. Psychiatry 26, 113-130.

Avissar, S., and Schreiber, G. (1992) The involvement of guanine nucleotide binding proteins in the pathogenesis and treatment of affective disorders. Biol. Psychiatry 31, 435-459.

Backheit, A.M., Behan, P.O., Dinan T.G., Gray, C.E., O'Keane, V. (1992) Possible upregulation of hypothalamus 5-Hydroxytryptamine receptors in patients with postviral fatigue syndrome. Brit. Med. J. 304, 1010-1012.

Bell, I.R., Miller, C.S., and Schwartz, G.E. (1992) An olfactory-limbic model of multiple chemical sensitivity syndrome: Possible relationships to kindling and affective spectrum disorders. Biol. Psychiatry 32, 218-242.

Benca, R.M., Obermeyer, W.H. Thisted, R.A., and Gillin, J.C. (1992) Sleep and psychiatric disorders: A meta-analysis. Arch. Gen. Psychiatry 49, 651-670.

Black, D.W., Rathe, A., and Goldstein, R.B. (1990) Environmental illness. A controlled study of 26 subjects with "20th Century Disease". JAMA 264, 166-170.

Breslau, N., Kilbey, M.M., and Andreski, P. (1991) Nicotine dependence, major depression and anxiety in young adults. Arch. Gen. Psychiatry 48, 1061-1074.

Bushnell P.J., Levin, E.D., Overstreet, D.H. (1995) Spatial working and reference memory in rats bred for autonomic sensitivity to cholinergic stimulation: Acquisition, accuracy, speed, and effects of cholinergic drugs. Neurobiology of Learning and Memory 63, 116-132.

Chaudhuri A., Majeed T., Dinan T., Behan P.O. (1997) Chronic fatigue syndrome: A disorder of central cholinergic transmission. J. Chronic Fatigue 3, 3-16.

Cone, J.E. and Sult, T.A. (1992) Acquired intolerance to solvents following pesticide/solvent exposure in a building: a new group of workers at risk for multiple chemical sensitivities? Toxicol. Indust. Health 8, 29-39.

Cox, B., Kerwin, R.W., Lee, T.F., and Pycock, C.J. (1980) A dopamine-5-Hydroxytryptamine link in the hypothalamic pathways which mediate heat loss in the rat. J. Physiol. 303, 9-21.

Criswell, H.A., Overstreet, D.H., Rezvani, A.H., Johnson, K.B., Simson, P.E., Knapp, D.J., Moy, S.S., and Breese, G.R. (1994) Effects of ethanol, MK-801, and chlordiazepoxide on locomotor activity in different rat lines: Dissociation of locomotor stimulation from ethanol preference. Alcohol. Clin. Exp. Res. 18, 917-923.

Crocker A.D., and Overstreet, D.H. (1991) Changes in dopamine sensitivity in rats selectively bred for differences in cholinergic function. Pharmacol. Biochem. Behav. 38, 105-108.

Cullen, M.R. (1987) Workers with multiple chemical sensitivities. Occup. Med. State Art Rev. 2, 655-806.

Daws, L.C., Schiller, G.D., Overstreet, D.H., Orbach, J. (1991) Early development of muscarinic supersensitivity in a genetic animal model of depression. Neuropsychopharmacology 4, 207-217.

Fibiger, H.C., Lytle, L.D., and Campbell, B.A. (1970) Cholinergic modulation of adrenergic arousal in the developing rat. J. Comp. Physiol. Psychol. 3, 384-389.

Fiedler, N., Maccia, C., and Kipen, H. (1992) Evaluation of chemicaly sensitive patients. J. Occup. Med. 34, 529-538.

Friedman, A., Kaufer D., Shemer, J., Hendler, I., Soreq, H., Tur-Kaspar, I. (1996) Pyridostigmine brain penetration under stress enhances neronal excitability and induces immediate transcriptional response. Nature Med. 2, 1382-1385.

Gann, H., Riemann, D., Hohagen, F., Dressing, H., Muller, W.E., and Berger, M. (1992) The sleep structure of patients with anxiety disorders in comparison to that of healthy controls and depressive patients under baseline conditioons and after cholinergic stimulation. J. Affect. Dis. 26, 179-190.

Gershon, S. and Shaw, F.H. (1961) Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1,1371-1374.

Ghigo, E., Nicolosi, M., Arvat, E., Marcone, A., Danelon, F., Mucci, M., Franceschi, M., Smirne, S., and Camanni, F. (1993) Growth hormone secretion in Alzheimer's disease: studies with growth hormone-releasing hormone alone and combined with pyridostigmine or arginine. Dementia 4, 315-320.

Gillin, J.C., Sutton, L., Ruiz, C., Kelsoe, J., Dupont, R.N., Darko, D., Risch, S.C., Golshan, S., and Janowsky, D. (1991) The cholinergic rapid eye movement induction test with arecoline in depression. Arch. Gen. Psychiatry 48, 264-270.

Glassman, A.H. (1993) Cigarette smoking: Implications for psychiatric illness. Am. J. Psychiatry 150, 546-553.

Goodwin, F.K. and Jamison, K.R. (1990) Manic-Depressive Illness, Oxford University Press, New York.

Gupta, R.P. and Abou-Donia, M.B. (1994) In vivo and in vitro effects of diisopropyl phosphofluoridate (DFP) on the rate of brain tubulin polymerization. Neurochem. Res. 19, 435-444.

Huff, R.A., Corcoran, J.J., Anderson, J.K., and Abou-Donia, M.B. (1994) Chlorpyrifos oxon binds directly to muscarinic receptors and inhibits cAMP accumulation in rat striatum. J. Pharmacol. Exp. Ther. 269, 329-335.

Janowsky, D.S. and Risch, S.C. (1987) Acetylcholine mechanisms in affective disorders. In: H.Y. Meltzer (Ed) Psychopharmacology. The Third Generation of Progress, Raven Press, New York, pp. 527-534.

Janowsky, D.S., El-Yousef, M.K., Davis, J.M., and Sekerke, H.J. (1972) A cholinergic-adrenergic hypothesis of mania and depression. Lancet 2, 632-635.

Janowsky, D.S., Overstreet, D.H., and Nurnberger J.I.Jr. (1994) Is cholinergic sensitivity a genetic marker for the affective disorders? Am. J. Med. Genet. (Neuropsychiatric Genetics) 54, 335-344.

Kendler, K.S., Heath, A.C., Neale, M.C., Kessler, R.C., and Eaves, L.J. (1993) Alcoholism and major depression in women. A twin study of the causes of comorbidity. Arch. Gen. Psychiatry 50, 690-698.

Klemm, W.R. (1989) Drug effects on active immobility responses: what they tell us about neurotransmitter systems and motor function. Prog. Neurobiol. 32, 403-422.

Kupfer, D.J. (1976) REM latency. A psychobiological marker for primary depressive disease. Biol. Psychiatry 11, 159-174.

Lesch, K.P. and Manji, H.K. (1992) Signal-transducing G proteins and antidepressant drugs: evidence for modulation of alpha-subunit gene expression in rat brain. Biol. Psychiatry 32, 549-579.

Lesch, K.P., Disselkamp-Tietze, J., and Schmidtke, A. (1990) 5-HT1A receptor function in depression: Effect of chronic amitriptyline treatment. J. Neural Transm. 80, 157-161.

Lucey, J.V., Butcher, G., Clare, A.W., and Dinan, T.G. (1993) Elevated growth hormone responses to pyridostigmine in obsessive-compulsive disorder: evidence of cholinergic supersensitivity. Am. J. Psychiatry 150, 961-962,

Maier, W., Lichtermann, D., and Minges, J. (1994) The relationship between alcoholism and unipolar depression - a controlled family study. J. Psychiatry Res. 28, 303-316.

Martin, J.D., Durand, D., Gurd, W., Faille, G., Audet, J., and Brazeau, P. (1978) Neuropharmacological regulation of episodic growth hormone-releasing hormone release into hypophysial portal blood of conscious sheep. Endocrinology 133, 1247-1251.

Mazza, E., Ghigo, E., Boffano, G., Valeto, M., Naccarioi, M., Arvat, D., Bellone, J., Procopio, M., Muller, E.E., and Camanni, F. (1994) Effects of direct and indirect acetylcholine receptor agonists on growth hormone secretion in humans. Eur. J. Pharmacol. 254, 17-20.

Meltzer, H.Y., and Lowy, M.T. (1987) The serotonin hypothesis of depression. In Psychopharmacology: The Third Generation of Progress. In: H.Y. Meltzer (Ed.), Raven Press, New York, pp. 513-526.

Mikuni, M., Kusumi, I., Kagaya, A., Kuroda, Y., Mori, H., and Takahashi, K. (1991) Increased 5-HT-2 receptor function as measured by serotonin-stimulated phosphoinositide hydrolysis in platelets of depressed patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 15, 49-62.

Miller, C.S. (1994) White paper: Chemical sensitivity: history and phenomenology. Toxicol. Indust. Health 10, 253-276.

Miller, C.S. and Mitzel, H.C. (1995) Chemical sensitivity attributed to pesticide exposure versus remodeling. Arch. Env. Health 50, 119-129.

Mitchell, F.L. and Price, P. (1994) Proceeding of the conference on low-level exposure to chemicals and neurobiologic sensitivity. Toxicol. Indust. Health 10, 1-300.

National Research Council (NRC) (1992) Multiple Chemical Sensitivities: Addendum to Biologic Markers in Immunotoxicology. National Academy Press, Washington, D.C.

Netherton, R.A. and Overstreet, D,H. (1983) Genetic and sex differences in the cholinergic modulation of thermoregulation. In: Environment, Drugs and Thermoregulation. Lomax P, Schonbaum E (Eds). Basel, Karger, pp. 74-77.

Nurnberger, J.I.Jr., Berrettini, W., Mendelson, W., Sack, D., and Gershon, E.S. (1989) Measuring cholinergic sensitivity: I. Arecoline effects in bipolar patients. Biol. Psychiatry 25, 610-617.

O'Keane, V., O'Flynn, K., Lucey, J., and Dinan, T.G. (1992) Pyridostigmine-induced growth hormone responses in healthy and depressed subjects - Evidence for cholinergic supersensitivity in depression. Psychol. Med. 22, 55-60.

O'Keane, V., Abel, K. and Murray, R.M. (1994) Growth hormone responses to pyridostigmine in schizophrenia: evidence for cholinergic dysfunction. Biol. Psychiatry 36, 582-586.

Overstreet, D.H. (1986) Selective breeding for increased cholinergic function: Development of a new animal model of depression. Biol. Psychiatry 21, 49-58.

Overstreet DH. (1989) Correlations of Ethanol-induced hypothermia in FSL and FRL rats with hypothermia induced by other drugs. Presented at 13th Annual Symposium of the North Carolina Alcoholism Research Authority, Raleigh.

Overstreet, D.H. (1993) The Flinders Sensitive Line rats: A genetic animal model of depression. Neurosci Biobehav Rev 17: 51-68.

Overstreet, D.H. and Janowsky, D.S. (1991) A cholinergic supersensitivity model of depression. In: A. Boulton, G. Baker, and M. Martin-Iverson, (Eds.) Neuromethods. Vol. 19: Animal Models in Psychiatry, II, Humana Press, Clifton, NJ, pp. 81-114.

Overstreet, D.H. and Measday, M. (1985) Impaired active avoidance performance in rats with cholinergic supersensitivity: Its reversal with chronic imipramine. Presented at 4th International Congress of Biological Psychiatry, Philadelphia, PA.

Overstreet, D.H. and Russell, R.W. (1982) Selective breeding for sensitivity to DFP. Effects of cholinergic agonists and antagonists. Psychopharmacology. 78, 150-154.

Overstreet D.H., Hadick, D.G., and Russell, R.W. (1972). Effects of amphetamine and pilocarpine on eating behavior in rats with chronically low acetylcholinesterase levels. Behav. Biol. 7, 212-226.

Overstreet, D.H., Kozar M.D., abd Lynch, G.D. (1973) Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. Neuropharmacology. 12, 1017-1032.

Overstreet, D.H., Russell, R.W., Helps, S.C., and Messenger, M. (1979) Selective breeding for sensitivity to the anticholinesterase, DFP. Psychopharmacology 65, 15-20.

Overstreet, D.H., Russell, R.W., Crocker, A.D., and Schiller, G.D. (1984) Selective breeding for differences in cholinergic function: Pre- and Post-synaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Research. 294, 327-332.

Overstreet, D.H., Booth, R., Dana, R., Risch, S.C., and Janowsky, D.S. (1986a) Enhanced elevation of corticosterone following arecoline administration to rats selectively bred for increased cholinergic function. Psychopharmacology 88, 129-130.

Overstreet, D.H., Janowsky, D.S., Gillin, J.C., Shiromani, P., and Sutin, E.L. (1986b) Stress-induced immobility in rats with cholinergic supersensitivity. Biol, Psychiatry. 21, 657-664.

Overstreet, D.H., Russell, R.W., Crocker, A.D., Gillin, J.C., and Janowsky, D.S. (1988) Genetic and pharmacological models of cholinergic supersensitivity and affective disorders. Experientia 44, 465-472.

Overstreet, D.H., Double, K., and Schiller, G.D. (1989a) Antidepressant effects of rolipram in a genetic animal model of depression: Cholinergic supersensitivity and weight gain. Pharmacol. Biochem. Behav. 34, 691-696.

Overstreet, D.H., Janowsky, D.H., and Rezvani, A.H. (1990a) Impaired active avoidance responding in rats selectively bred for increased cholinergic function. Physiol. Behav. 47, 787-788.

Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1990b) Increased hypothermic responses to ethanol in rats selectively bred for cholinergic supersensitivity. Alcohol & Alcohol.. 25, 59-65.

Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1992a) Genetic animal models of depression and ethanol preference provide support for cholinergic and serotonergic involvement in depression and alcoholism. Biol. Psychiatry 31, 919-936.

Overstreet, D.H., Janowsky, D.S., Pucilowski, O., and Rezvani, A.H. (1994) Swim test immobility cosegregates with serotonergic but not cholinergic sensitivity in cross breeds of Flinders Line rats. Psychiat. Genet. 4, 101-107.

Overstreet, D.H., Pucilowski, O., Rezvani A.H., and Jaowsky, D.S., (1995) Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. Psychopharmacology 121, 27-37.

Overstreet D.H., Miller, C.M., Janowsky, D.S., Russell, R.W. (1996) A potential animal model of multiple chemical sensitivity with cholinergic supersensitivity. Toxicology 111, 119-134.

Overstreet, D.H., Rezvani, A.H., Yang Y., Hamedi H., Janowsky, D.S. (1997) Animal model of chemical sensitivity involving cholinergic agents. Presented at Toxicology in Risk Assessment Symposium held in Bethesda, MD, May 14-16, 1997.

Pepe, S., Overstreet, D.H., and Crocker, A.D. (1988) Enhanced benzodiazepine responsiveness in rats with increased cholinergic function. Pharmacol. Biochem. Behav. 31, 15-20.

Pucilowski, O. (1987) Monoaminergic control of affective aggression. Acta Neurobiol. Exp. 47, 25-50.

Pucilowski, O. and Overstreet, D.H. (1993) Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. Pharmacol. Biochem. Behav. 32, 471-475.

Pucilowski, O., Danysz, W., Overstreet, D.H., Rezvani, A.H., Eichelman, B., and Janowsky, D.S. (1991a) Decreased hyperthermic effect of MK-801 in selectively bred hypercholinergic rats. Brain Res. Bull. 26, 621-525.

Pucilowski, O., Eichelman, B.S., Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1991b) Enhanced affective aggression in genetically bred hypercholinergic rats. Neuropsychobiology. 24, 37-41.

Pucilowski, O., Overstreet, D.H., Rezvani, A.H., and Janowsky, D.S. (1993). Chronic mild stress-induced anhedonia: Greater effect in a genetic rat model of depression. Physiol. Behav. 54, 1215-1220.

Ray, A., Sen, P., and Alkondon, M. (1989) Biochemical and pharmacological evidence for central cholinergic regulation of shock-induced aggression. Pharmacol. Biochem. Behav. 32, 867-871.

Rezvani, A.H., Overstreet, D.H., Ejantkar, A., and Gordon, C.J. (1994) Autonomic and behavioral responses of selectively bred hypercholinergic rats to oxotremorine and diisopropyl fluorophosphate. Pharmacol. Biochem. Behav. 48, 703-707.

Rosenstock, L., Keifer, M., Daniell, W., McConnell, R., and Claypoole, K. (1991) Chronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 338, 223-227.

Rosenthal, N. and Cameron, C.L. (1991) Exaggerated sensitivity to an organophosphate pesticide (letter). Am. J. Psychiatry 148, 270.

Rowntree, D.W., Neven, S., and Wilson, A. (1950) The effect of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. J. Neurol. Neurosurg. Psychiat. 13, 47-62.

Russell, R.W. and Overstreet, D.H. (1987) Mechanisms underlying sensitivity to organophosphorus anticholinesterase agents. Prog. Neurobiol. 28, 97-129.

Russell, R.W., Overstreet, D.H., Messenger, M., and Helps, S.C. Selective breeding for sensitivity to DFP. Generalization of effects beyond criterion variables. Pharmacol. Biochem. Behav. 17:885-891, 1982.

Savage, E.P., Keefe, T.J., and Mounce, L.M. (1988) Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch. Environ. Health 43, 38-45.

Schiller, G.D., and Overstreet, D.H. (1993) Selective breeding for increased cholinergic function: Preliminary study of nicotinic mechanisms. Medic. Chem. Res. 2, 578-583.

Schiller, G.D., Orbach, J., and Overstreet, D.H. (1988) Effects of intracerebroventricular administration of site selective muscarinic drugs in rats genetically selected for differing cholinergic sensitivity. Presented at meeting of Australasian Society for Clinical and Experimental Pharmacology, Adelaide, December.

Schiller, G.D., Daws, L.C., Overstreet, D.H., Orbach, J. (1991) Absence of anxiety in an animal model of depression with cholinergic supersensitivity. Brain Res. Bull. 26, 443-447.

Schiller, G.D., Pucilowski, O., Wienicke, C., and Overstreet, D.H. (1992) Immobility-reducing effect of antidepressants in a genetic animal model of depression. Brain Res. Bull. 28, 821-823.

Schreiber, W., Lauer, C.J., Krumrey, K., Holsboer, F., and Krieg, J.C. (1992) Cholinergic REM sleep induction test in subjects at high risk for psychiatric disorders. Biol. Psychiatry 32, 79-90.

Schuckit, M.A. (1986) Genetic and clinical implications of alcoholism and affective disorders. Am. J. Psychiatry 143, 140-147.

Shiromani, P.J., Gillin, J.C., and Hendrickson, P. (1987) Acetylcholine and the regulation of REM sleep - Basic mechanisms and clinical implications for affective illness and narcolepsy. Annu. Rev. Pharmacol. Toxicol. 27, 137-156.

Shiromani, P.J., Overstreet, D.H., Levy, D., Goodrich, C.A., Campbell, S.S., and Gillin, J.C. (1988) Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropsychopharmacology 1, 127-133

Sihotang, K. and Overstreet, D.H. (1983) Studies on the possible relationship of brain proteins to behavioral sensitivity to DFP. Life Sci. 32, 413-420.

Simon, G.E., Katon, W.J., and Sparks, P.J. (1990) Allergic to life: Psychological factors in environmental illness. Am. J. Psychiatry 147, 901-906.

Sitaram, N., Jones, D., Dube, S., Keshavan, M., Bell, J., Davies, A., and Reynal, P. (1987) The association of supersensitive cholinergic-REM induction and affective illness within pedigrees. J. Psychiatry Res. 21, 487-497.

Tabershaw, I.R. and Cooper, C. (1966) Sequelae of acute organic phosphate poisoning. J. Occup. Med. 8, 5-20.

Wachtel, H. (1989) Dysbalance of neuronal second messenger function in aetiology of affective disorders: A pathophysiological concept hypothesizing defects beyond first messenger receptors. J. Neural Transm. 75, 21-29.

Wallis, E., Overstreet, D.H., and Crocker, A.D. (1988) Selective breeding for increased cholinergic function: Increased serotonergic sensitivity. Pharmacol. Biochem. Behav. 31, 345-350.

Documentation of Personnel and Bibliography

Personnel Involved in the Project

David H. Overstreet, Ph.D., Principal Investigator
Amir H. Rezvani, Ph.D., Co-Investigator
Ying Yang, M.D., Research Associate
Elijah Clark, Jr., Research Assistant

Bibliography

Overstreet, D.H., Rezvani, A.H., Yang Y., Hamedi H., Janowsky, D.S. (1997) Animal model of chemical sensitivity involving cholinergic agents. Presented at Toxicology in Risk Assessment Symposium held in Bethesda, MD, May 14-16, 1997. (Manuscript in Appendix to Annual Report).

Overstreet, D.H., Yang Y, Hamedi, M., Janowsky, D.S., Rezvani, A.H. Strain- and Gender-Dependent Effects of Oxotgremorien and Pyridostigmine. To be presented at Society for Neuroscience, New Orleans, October, 1997. (Abstract attached).

SOCIETY FOR NEUROSCIENCE
1997 ABSTRACT FORM

Read all instructions before typing abstract.
See *Call for Abstracts* and reverse of this sheet.
Complete abstract and **all boxes**
at left and below before making copy
(Please type or print in black ink.)

_______ Check here if this is a REPLACEMENT of abstract submitted earlier. Remit a nonrefundable **$40** for each replacement abstract. Replacement abstracts must be RECEIVED by Tuesday, May 6, 1997.

First (Presenting) Author

Provide full name (no initials), address, and phone numbers *of first author on abstract. You may present (first author) only one abstract.* (Please type or print in black ink.)

David H. Overstreet
Center for Alcohol Studies
3011 Thurston-Bowles Bldg. CB 7178
Univ. of North Carolina at Chapel Hill
NC 27599-7178 Fax: (919) 966-5679
Office: (919 966-5678 Home: (919)968-1939
E-mail: dhover.@med.unc.edu

SMALLEST RECOMMENDED TYPE SIZE: 10 POINT

SAMPLE:
1997 Annual Meeting
New Orleans, La.
Oct. 25–30, 1997

POSTMARK DEADLINE:

FRIDAY, APRIL 25, 1997

An asterisk must be placed after the sponsor's (signing member) name on the abstract.

Presentation Preference

Check one: ☒ poster ☐ slide

Themes and Topics

See list of themes and topics, pp. 17–18. Indicate below a first and second choice appropriate for programming and publishing your paper.

1st theme title: Disorders of the Nervous System **theme letter:** J
1st topic title: Genetic Models **topic number:** 134

2nd theme title: Disorders of the Nervous System **theme letter:** J
2nd topic title: Neurotoxicity **topic number:** 151

Special Requests (for example, projection, video, or computer requirements)

Include nonrefundable **abstract handling fee** of **$40**. Fill out payment information form below. Purchase orders will not be accepted. Submission of abstract handling fee does not include registration for the Annual Meeting.

STRAIN- AND GENDER-DEPENDENT EFFECTS OF OXOTREMORINE AND PYRIDOSTIGMINE. D.H. Overstreet*, Y. Yang, M. Hamedi, D.S. Janowsky and Amir H. Rezvani, Skipper Bowles Ctr. for Alcohol Studies, UNC, Chapel Hill, NC 27599-7178.

Pyridostigmine (PYR) was given to many participants in the Gulf War as a protection against exposure to nerve gases and some individuals reported side effects. To test the hypothesis that a subset of individuals may have been genetically supersensitive, the effects of PYR were determined in both genders of three strains of rats: The Flinders Sensitive Line (FSL), the Flinders Resistant Line (FRL) and Sprague-Dawley (SD). Rats were first challenged with a mixture of methyl atropine (2 mg/kg) and oxotremorine (OX - 0.2 mg/kg) and temperatures telemetrically monitored to measure cholinergic sensitivity. Females of each strain exhibited approximately 0.6 ^{o}C greater decreases in temperature than their male counterparts. The FSL rats were the most sensitive to OX, as expected, and the SD rats were intermediate between the FSL and FRL rats, confirming that both the FSL and FRL rats have diverged from normal SD rats. PYR, up to a dose of 12 mg/kg orally, did not produce a decrease in temperature in any of the 6 groups, confirming the lack of central effects. However, the FSL male rats appeared to be more sensitive to the heart rate lowering effects of PYR, a finding consistent with increased peripheral cholinergic sensitivity. Data on blood growth hormone levels, known to be stimulated by PYR, are being analyzed and will be reported at the meeting. These findings are consistent with the view that some humans may be genetically predisposed to exhibit greater sensitivity to PYR. (Supported by U.S. Army).

Key Words: (see instructions p. 4)

1. Cholinergic Sensitivity
2. Flinders Rats
3. Temperature
4. Growth Hormone

Signature of Society for Neuroscience member required below. ***No member may sign more than one abstract. The signing member must be an author on the paper and an asterisk must be placed after the sponsor's (signing member) name on the abstract.***

The signing member certifies that any work with human or animal subjects related in this abstract complies with the guiding policies and principles for experimental procedures endorsed by the Society. This signature acknowledges that each author on this abstract has seen and approved the final version of the abstract and has given consent to appear as an author. Abstracts must comply with ethical guidelines for human and animal research, and authors may be asked to supply added documentation.

Society for Neuroscience member's signature

David H. Overstreet
Printed or typed name

(919)966-1159
Telephone number